Table 1. Amino-terminal sequence of LH-RH–induced pituitary protein (LHRH70A) and its acidic isoform (LHRH70B) and estrogen-induced brain protein (EI70A) and its acidic isoform (EI70B). For sequencing, the proteins were induced as in Fig. 1 and text. The VMH proteins from eight rats were separated on eight two-dimensional gels, transferred from two-dimensional gels to a polyvinyldifluoride membrane (Millipore), and stained as in Fig. 1. These proteins were then cut out of the membrane (boxed in Fig. 1), and spots from eight membranes were pooled and subjected to microsequencing by sequential Edman degradation on a gas-phase sequencer (Applied Biosystems, model 470A). Edman degradation cycles were continued at least five times or until cycle-specific amino acid peaks became ambiguous. X indicates an ambiguous amino acid peak.

Protein	Sequence
LHRH70A	NH_2-DVLELTDENFESRVSDTG
EI70A	NH_2-XVLELTDENFESXVSXTG
LHRH70B	NH_2-XVLELTDENXESRVSXXX
EI70B	NH_2-XVLELTXENFEXXXXXXX

by increasing the transcription or activity, respectively, of a common phosphatase (*12*) that would dephosphorylate the acidic isoforms EI70B and LHRH70B. For example, estrogen decreases the phosphorylation of a uterine protein whose phosphorylation is also regulated by adenosine 3′,5′-monophosphate (cAMP) (*13*), LH-RH–stimulated LH release may be potentiated by cAMP (*14*), peptide hormones can cause dephosphorylation through a cAMP-dependent mechanism (*15*), and there is a direct quantitative relation between the dephosphorylation of a specific protein, myosin, and the release of the neurotransmitter serotonin (*16*). The HIP-70 may represent the first example of a steroid hormone and a peptide hormone acting to decrease phosphorylation of the same protein in a common target tissue. Thus estrogen and LH-RH could each contribute to the dephosphorylation of EI70B or LHRH70B (leading to increased HIP-70) and synergistically amplify each other's ability to enhance neuroendocrine secretion. In this study HIP-70 was induced by LH-RH in pituitaries of intact proestrous rats, in which ovarian estrogen was rising but endogenous LH-RH was still too low to induce the LH surge or pituitary sensitization to LH-RH (self-priming) (*3*). It remains to be determined if LH-RH could induce HIP-70 in the absence of estrogen.

HIP-70 appears to be a novel protein. Further work will be necessary to determine the physiological function of HIP-70 and whether dephosphorylation of EI70B and LHRH70B leads to enhanced neuroendocrine secretion, but our studies have indicated the utility of combining two-dimensional gel electrophoresis with a microsequencing procedure to analyze hormone regulation in complex protein mixtures (*10*).

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Isolation of a G Protein That Is Modified by Learning and Reduces Potassium Currents in *Hermissenda*

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In *Hermissenda crassicornis* conditioned to associate light and rotation, type B photoreceptor neurons exhibit pairing-specific decreases in the potassium currents I_A and $I_{K\text{-}Ca}$, which account for many of the behavioral changes elicited by associative conditioning. To determine which proteins are involved in storage of this memory, high-performance liquid chromatography was used to examine proteins from *Hermissenda* eyes. Conditioning-specific changes in four phosphoproteins were observed 24 hours after conditioning. One of these proteins, cp20, was purified to apparent homogeneity and found to be a G protein. When injected back into *Hermissenda* type B cells, cp20 reduced I_K and $I_{K\text{-}Ca}$ in a manner indistinguishable from the reduction caused by conditioning, suggesting that this protein may play a crucial role in memory acquisition or retention.

IN THE TYPE B PHOTORECEPTOR NEURONS in the eye, associative conditioning of the marine nudibranch mollusk *Hermissenda crassicornis* with paired light and rotation causes a decrease in early K^+ (I_A) and Ca^{2+}-dependent K^+ currents ($I_{K\text{-}Ca}$) (*1*) and changes in the branching volumes of the terminal endings (*2*). Protein kinases and phosphoproteins may be involved at an early stage of these processes (*3*). For example, phosphorylation of a 20- to 21-kD protein from the *Hermissenda* eye is increased 3 hours after associative conditioning (*4*), perhaps mediated by protein kinase C (PKC) (*3, 5*). PKC, possibly together with Ca^{2+}-calmodulin–dependent kinase II, might phosphorylate this 20-kD protein, in turn producing the K^+ current changes induced by conditioning (*3, 5, 6*).

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To test these possibilities, *Hermissenda* were trained to associate light and rotation on a circular turntable (*7*), a procedure that induced a change in the phototactic response of the group exposed to paired light and rotation [latency ratio (latency in minutes after training/latency before training), 6.37 ± 1.56 (mean ± SEM, $n = 14$; $P < 0.001$; two-tailed t test)]. Animals receiving the same stimuli as the paired group, presented in a random temporal relationship, or animals receiving no light or rotation did not show a significant change [latency ratios, 1.33 ± 0.48 ($n = 12$) and 1.76 ± 1.47 ($n = 12$), respectively].

To examine how the proteins in *Hermissenda* neurons were affected by conditioning, individual eyes or circumesophageal ganglia (CNS) were isolated from trained, random, and naïve *Hermissenda* 24 hours after the end of training, and the proteins were analyzed by anion-exchange high-performance liquid chromatography (HPLC) (Fig. 1A).

The area (absorbance at 280 nm) of only

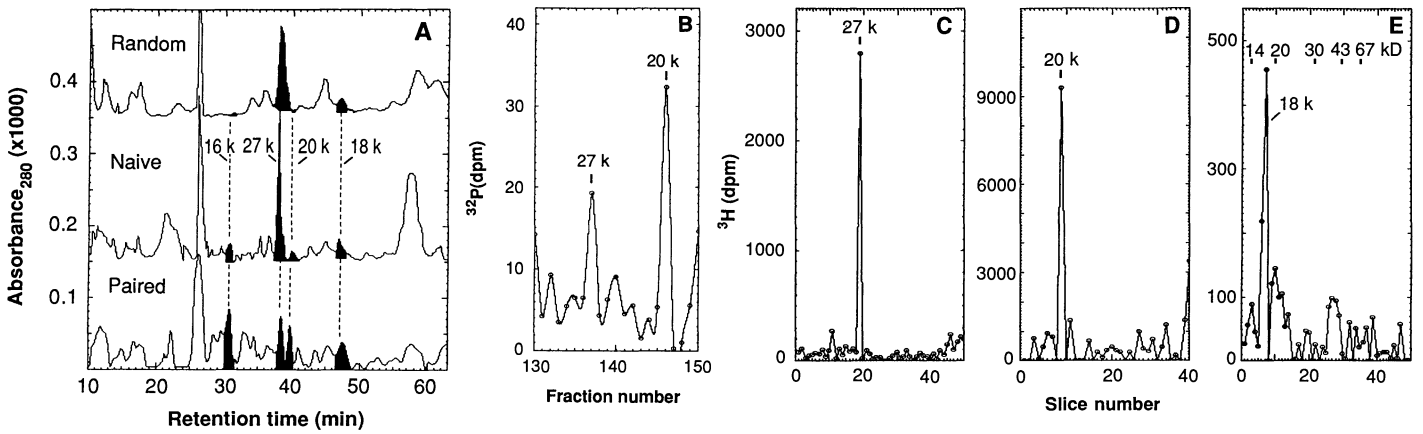

Fig. 1. (**A**) Typical HPLC tracings of proteins from eye of a *Hermissenda* subjected to random light and rotation (upper), a naïve animal (middle), or an animal subjected to light-rotation pairing (lower), prepared as in Table 1 and chromatographed on an ion-exchange (AX-300) column (0 to 0.6*M* sodium acetate, *p*H 7.4). Each chromatogram represents proteins from a single *Hermissenda* eye. A baseline (derived from a chromatogram with no injection) has been subtracted from each digitized chromatogram. Zero is set at 0.35 for the random tracing, 0.15 for naïve, and 0.0 for paired. Peaks with t_R <27 min were not analyzed. The peak with a t_R of 33.0 min was analyzed as the sum of two peaks. The peak at t_R 58.0 was variable among animals. About 80 to 85% of injected proteins eluted in the nonretained fraction between 0 and 10 min and between 25 and 28 min. (**B**) Identification of cp27 and cp20 as phosphoproteins. Seventeen naïve *Hermissenda* eyes were incubated at 15°C for 6 hours with 11 μCi of carrier-free [^{32}P]Pi, then homogenized, centrifuged (10,000*g*, 7 s), and chromatographed by AX-300 ion-exchange HPLC. Fractions (0.2 min) were collected and the radioactivity determined. Distinct peaks of ^{32}P incorporation, coinciding with the absorbance peaks, were visible for cp27 and cp20 in all chromatograms (n = 5). Fractions 137 and 146 correspond to peaks with retention times of 38.2 to 38.4 and 40.0 to 40.2 min, respectively. This experiment was done with a different AX-300 column than the other experiments in this paper. A blank of 35 dpm was subtracted from each data point. (**C** through **E**) SDS polyacrylamide gel profiles of conditioning-associated proteins (C) cp27, (D) cp20, and (E) cp18 from *Hermissenda* eyes after partial purification on ion-exchange HPLC. Fifteen naïve *Hermissenda* eyes were combined, homogenized, and chromatographed as in Fig. 1A. Fractions corresponding to each peak of interest were combined, desalted, lyophilized, dissolved in 2 μl of water, and incubated with 0.25 mCi of [^{3}H]acetic anhydride in 2 μl for 1 hour at 25°C (C and E) or 1.25 mCi in 10 μl of dimethyl sulfoxide (DMSO) for 18 hours (D) (*20*). Excess acetic anhydride was removed by lyophilization and samples were subjected to SDS-PAGE (10% acrylamide). Gel slices [2 mm for (C) and (E), 2.7 mm for (D)] were solubilized and the radioactivity determined. About 1×10^{-10} g of protein are present in (D) and (E), and 5×10^{-9} g in (C). Blanks of 40 to 50 dpm have been subtracted from (C) and (E) and 1000 dpm from (D).

four peaks was consistently changed after conditioning (Fig. 1A and Table 1). These peaks were designated cp20 [retention time (t_R), 39.8 min], which increased an average of 3.3-fold over the random group and 5.6-fold over the naïve group; cp16 (t_R, 30.8 min) and cp18 (t_R, 47.4 min), which increased 3.7- and 2.4-fold, respectively, compared with the random group; and cp27 (t_R, 38.3 min), which decreased by 68% compared to random (Table 1).

Incubation of isolated ganglia or eyes from naïve animals with [^{32}P]inorganic phosphate ([^{32}P]Pi) revealed that most peaks with t_R values between 28 and 60 min incorporated ^{32}P. In fact, all four proteins that were changed in absorbance after conditioning were phosphoproteins; cp20 incorporated 0.69 ± 0.25 mol of ^{32}P per mole of protein (mean ± SEM, n = 5) and cp27 incorporated 0.23 ± 0.14 mol of ^{32}P per mole of protein (n = 5) (Fig. 1B) (*8*).

Since anion-exchange HPLC separates phosphorylated and nonphosphorylated forms of proteins, an increase in absorbance peak area could be due to either more total protein or to a change in phosphorylation, which would shift more protein into the phosphorylated peak. To distinguish between these possibilities, we determined the effect of conditioning on the specific activity of the four conditioning-associated proteins after labeling with [^{3}H]amino acids (*9*). The specific activities of cp16, cp18, and cp20 were decreased, while that of cp27 was unchanged (Table 1). This result suggests that the changes in absorbance of cp16, cp18, and cp20 are not due to a long-term increase in the rate of protein synthesis, but rather to an increase in phosphorylation of the protein. (We cannot rule out the possibility that conditioning could cause a transient increase in cp20 synthesis that increases the amount of unlabeled protein

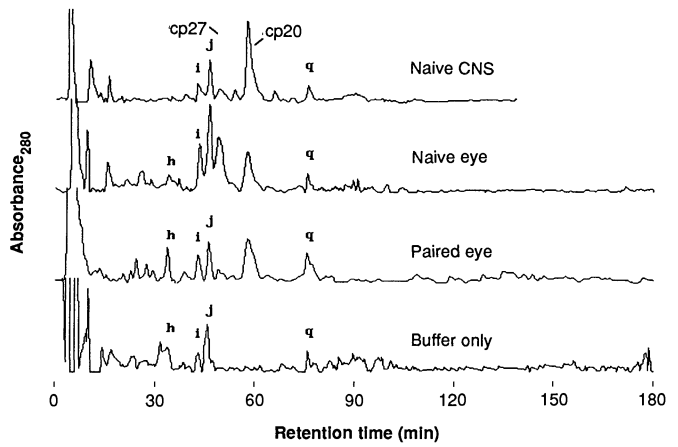

Fig. 2. Co-elution of cp20 from naïve CNS, naïve eyes, and paired eyes. Two naïve CNS's, 18 naïve eyes, or 38 paired eyes from *Hermissenda* were homogenized, centrifuged at 10,000*g*, and injected into an AX-300 HPLC ion-exchange column without freeze-thawing. Fractions corresponding to cp27 (117) and cp20 (121), and all intervening and adjacent fractions were desalted on a column of Sephadex G-25–150 (0.7 by 40 cm), partially lyophilized, injected into a C18 reversed-phase HPLC column and eluted with a gradient of 0.2% trifluoroacetic acid (TFA) in 20 to 100% acetonitrile in water (0 to 90 min) followed by 0.2% TFA in 100% acetonitrile (90 to 180 min). Cp27 eluted at about 48.2 min; cp20 eluted at 56.8 min. Although some cross-contamination from cp27 appears in the samples from naïve CNS and eye, no other protein peaks could be detected up to 3 hours. The recovery of cp20 was calculated by comparing the A_{280} peak areas for cp20 in the ion-exchange column with the A_{280} areas in the C18 column. The amounts of total protein injected were equal per injection [naïve CNS, 57% of total cp20 from two CNS's was injected; naïve eye, 100% of total from 18 eyes; paired eye, 16% of total from 38 eyes (0.0008 absorbance units full scale (AUFS), except upper curve which was 0.0016 AUFS]. Peaks h, i, j, and q were present in the buffer.

before the introduction of radioactivity, causing a decreased final specific activity.)

To determine the purity and molecular weight of the four conditioning-associated proteins, 15 to 20 pooled eyes or 2 to 3 CNS's from naïve *Hermissenda* were chromatographed on HPLC, and the fractions corresponding to cp27 (116–117), cp20 (120–121), and cp18 (143–145) were labeled with [^{3}H]acetic anhydride and analyzed by SDS–polyacrylamide gel electrophoresis (PAGE). Single identifiable peaks of radioactivity were observed (Fig. 1, C to E). In some cases, minor contaminants of adjacent HPLC peaks were present. The labeling of cp16 was insufficient to enable a confirmation of its molecular weight.

To further characterize the identity and purity of cp20, anion-exchange HPLC fractions containing cp20 were reinjected on a C18 reversed-phase HPLC column. About 88 to 95% of the cp20 from the anion-exchange column was recovered as a single peak with a t_R of ≈56.8 (Fig. 2). In this experiment, conditioning increased the amount of cp20 by 3.3-fold in conditioned eyes compared to naïve eyes, from 76.8 pg of protein per eye in naïve to 254 pg of protein per eye in conditioned animals. Thus, the cp20 peaks in Fig. 1A consist of a single protein that is at least 88% pure, and the increase in cp20 after conditioning is not due to the appearance of a new protein, but to an increase in the amount of cp20. It also confirms that the cp20 proteins isolated from conditioned eyes, naïve eyes, and total CNS are identical.

Since K^+ channels are altered by conditioning in *Hermissenda* (*1*), as well as by some G proteins (*10*), we tested the conditioning-associated proteins for guanosine triphosphate (GTP)–binding and GTPase activity. For cp20, purified from *Hermissenda* CNS by anion-exchange and size-exclusion HPLC, a single peak containing protein, GTPase activity, and GTP-binding activity was detected, suggestive of a G protein (Fig. 3). The turnover rate of the GTPase was estimated to be about 0.1 min^{-1}, a value similar to that of other G proteins (*11, 12*). The

Table 2. Effect of cp20 injection on I_A and $I_{K\text{-}Ca}$. Mean ± SEM of peak current amplitudes. Methods as in Fig. 4. Data were analyzed by a two-way repeated measures ANOVA and subsequent pair comparison tests.

	cp20 (n = 7)	Heat-inact. cp20 (n = 7)	KAc (n = 7)
I_A			
Before	44.9 ± 2.9	44.1 ± 4.5	45.1 ± 3.0
After	22.5 ± 3.6*	45.4 ± 4.5	45.7 ± 3.1
$I_{K\text{-}Ca}$			
Before	24.4 ± 2.8	24.1 ± 4.5	22.1 ± 4.1
After	7.5 ± 1.1*	24.5 ± 2.8	22.6 ± 4.2

*P < 0.001, by two-factor ANOVA with one repeated measure.

Table 1. Effect of conditioning on HPLC peak absorbance areas and specific activities of proteins from *Hermissenda* eye. Individual *Hermissenda* eyes were dissected at 15°C 24 hours after the end of training (7) and incubated at 15°C for 6 hours in complete darkness in 12 μCi of mixed [^{3}H]amino acids in artificial sea water (ASW). The eyes were then rinsed with ASW, homogenized in a micro Dounce homogenizer in 20 μl of 10 m*M* tris (*p*H 7.6), 10 μg/ml leupeptin, 10 μg/ml pepstatin, 0.1 m*M* phenylmethylsulfonyl fluoride (PMSF), 50 m*M* NaF, 1 m*M* EDTA, and 1 m*M* EGTA. The homogenate was freeze-thawed (−70°C) and chromatographed on an AX-300 HPLC column without centrifugation (as in Fig. 1). Fractions (0.3 min) were collected and analyzed by scintillation spectrometry. Specific activities were calculated from peak areas in pairs of chromatograms ($^3H/A_{280}$). Peak areas were analyzed as percentage of the total retained peak area. All values are average ± SEM from chromatograms of proteins from individual eyes, which consist of about 0.1 to 0.2 μg of tissue and contain five neurons, two to three small pigmented cells, a small lens, and two epithelial cells. Retention times correspond to those in the lower curve of Fig. 1A. Peak areas measured blindly by hand (height × width at half height) were in close agreement (±10%) with those calculated by an electronic integrator. For some samples, the chromatograms were also digitized and the peak areas computed after Fourier deconvolution with a Gaussian response function. Except for the broad peak at a t_R of 57.5 min, these areas were also within 10% of those measured by hand. No consistent changes in peak widths were observed in any experiments. Only the peaks showing a change in ^{3}H incorporation with conditioning are shown in the lower section; the remaining 13 peaks showed no significant between-group differences.

Peak	t_R (min)	M_r (×10^{-3})	Random (n = 14)	Paired (n = 14)	Naïve (n = 12)	Paired/ random
			Absorbance$_{280}$ (% total area)			
	27.2		0.87 ± 0.37	0.59 ± 0.17	1.15 ± 0.35	0.68
	28.3		1.03 ± 0.59	0.75 ± 0.49	0.40 ± 0.16	0.73
	29.4		0.30 ± 0.09	1.45 ± 0.63*	0.50 ± 0.17	4.83
cp16	30.8	16	0.29 ± 0.06	1.06 ± 0.30**	0.29 ± 0.11	3.67
	33.1	27	2.94 ± 1.00	1.78 ± 1.56	1.55 ± 0.52	0.61
	34.4	11	1.37 ± 0.32	1.35 ± 0.40	1.12 ± 0.45	0.99
	36.5	7	0.78 ± 0.26	1.71 ± 0.55	0.71 ± 0.53	2.18
cp27	38.3	27	10.84 ± 1.58	3.48 ± 0.73***	9.08 ± 1.07	0.32
cp20	39.8	20	0.37 ± 0.09	1.23 ± 0.27**	0.22 ± 0.11	3.28
	41.1	38	0.80 ± 0.35	0.47 ± 0.11	0.98 ± 0.21	0.59
	43.5		2.46 ± 0.79	2.33 ± 0.52	0.69 ± 0.06†	0.95
	45.2		3.30 ± 1.60	6.52 ± 2.06	11.20 ± 5.50	1.98
cp18	47.4	18	0.84 ± 0.26	2.02 ± 0.47*	0.78 ± 0.22	2.40
	49.6		0.22 ± 0.06	0.76 ± 0.30	0.50 ± 0.24	3.45
	52.8		0.98 ± 0.55	1.57 ± 0.60	1.74 ± 0.56	1.60
	57.5		2.40 ± 1.20	2.59 ± 0.90	4.34 ± 1.80	1.08
	63.2		2.18 ± 0.82	2.36 ± 1.50	2.86 ± 1.22	1.08
			^{3}H-specific activity (arbitrary units)			
cp16	30.8		1670 ± 470	382 ± 103*	1120 ± 700	0.23
cp27	38.3		30 ± 16	44 ± 10	17 ± 5	1.46
cp20	39.8		590 ± 203	190 ± 44*	1070 ± 380	0.33
cp18	47.4		455 ± 130	210 ± 50*	370 ± 110	0.46

*P < 0.05. **P < 0.01. ***P < 0.001 (two-tailed t test for both paired versus random and paired versus naïve; not significant for random versus naïve). Cp16, cp20, and cp27 were significantly affected by conditioning (P < 0.05, P < 0.005, and P < 0.01, respectively) in a multivariate analysis (MANOVA). Subsequent two-tailed t tests on individual peaks revealed that all of the differences were in the paired group, with one exception (t_R = 43.5). †Different from paired and random groups at P < 0.01.

[^{35}S]GTPγS binding of cp20 corresponds to a stoichiometry of about 0.3 to 0.4 mol of [^{35}S]GTPγS per mole of protein (*13*). Both GTPase and GTP-binding activities of cp20 were a small proportion of the total activity in the CNS homogenate (about 1.0% for GTPase and 0.3% for GTP binding), and the protein was about 0.05% of the total CNS protein. No GTP-binding activity comigrated with the other conditioning-associated proteins (cp16, cp18, or cp27).

Since cp20 bound a minimum of 0.3 to 0.4 mol of GTPγS per mole of protein, any G protein contaminating cp20 would have to be at least 30% of the total on a molar basis to account for our results. Analysis for contaminating proteins with reversed-phase HPLC (Fig. 2), size-exclusion HPLC (Fig. 3), and SDS-PAGE (Fig. 1C), failed to detect any contaminants at levels >10% (the limit of detectability) other than cp27, which was not a G protein. Also, since the protein was about 88% pure, 30% contamination with any other protein would be unlikely. Thus, the GTP-binding activity probably resides in cp20 itself.

To test whether cp20 affected the K^+ currents that are reduced after conditioning, we iontophoretically injected purified cp20 into isolated *Hermissenda* type B photoreceptors through one of the two microelectrodes (that used for voltage measurement) used to voltage clamp the cell body. Injection of cp20 reduced both early (I_A) and late ($I_{K\text{-}Ca}$) K^+ currents (Fig. 4). Since the electrode contained about 0.09 μg of cp20, one can estimate (assuming, as an upper limit, equal mobilities of acetate and cp20) that a maximum of 3.0×10^{-14} g of cp20 could have been injected over 2 min. Injections of control solutions alone (1*M* KAc that had been carried through the HPLC and concentration steps or heat-inactivated cp20 in 1*M* KAc) had no effect on the K^+ currents (Fig. 4). Injection of another randomly selected ion-exchange HPLC peak fraction also had no effect [I_A before, 45.0 ± 0.7 nA; I_A after, 45.7 ± 0.5 (n = 3); $I_{K\text{-}Ca}$ before, 23.0 ± 1.9 nA; $I_{K\text{-}Ca}$ after, 24.0 ± 1.7 (n = 3)]. There were no changes in the light-induced Na^+ current or in membrane resting potential after injection of cp20.

Although changes in proteins have been shown to occur after operant-type learning (*14*) or long-term facilitation (*15*), associative conditioning of *Hermissenda* causes four protein changes that are localized to a few calls and are attributable to the temporal association between the stimuli. One of these proteins, cp20, is particularly interesting in that a similar or identical protein is phosphorylated after conditioning (*4*), it has properties of a small molecular weight G protein, and it can mimic the effects of associative conditioning on K^+ currents in cells previously implicated in memory storage. Cp20 may therefore be directly involved in memory acquisition or consolidation.

The biochemical identification of cp20 as a G protein is consistent with its physiological effect, that is, blockade of a K^+ channel. G proteins can directly or indirectly modu-

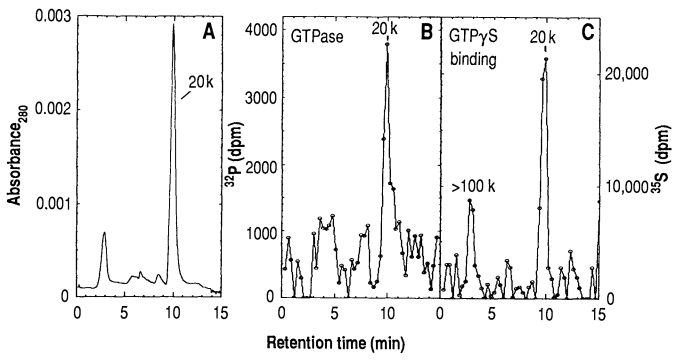

Fig. 3. Size-exclusion HPLC (**A**) absorbance profile, associated (**B**) GTPase, and (**C**) [^{35}S]GTPγS-binding activity of cp20. Circumesophageal ganglia from 20 naïve *Hermissenda* were homogenized in buffer and centrifuged for 7 s at 10,000*g*. The supernatant was injected (without freeze-thawing) into an AX-300 HPLC column and eluted at 10°C with a gradient of 0 to 0.6*M* KAc, *p*H 7.4. Fractions containing cp20 were then desalted by Sephadex G-25–150 chromatography, concentrated to 20 μl by partial lyophilization, and rechromatographed on a size-exclusion HPLC column (GPC-100) at 10°C in 0.1*M* KAc, *p*H 7.4. Fractions (0.3 min) were collected and assayed for enzyme or binding activity. Each panel represents a separate, parallel experiment and is typical of eight GTPase and three GTPγS binding experiments. The A_{280} peak, GTPase, and GTPγS-binding activity in each case eluted in one or two fractions, with average t_R's of 9.7 ± 0.1 min (n = 11), 9.8 ± 0.2 (n = 8), and 9.8 ± 0.5 min (n = 3), respectively, and average widths of 0.67 ± 0.05 min (n = 11), 0.66 ± 0.08 (n = 8), and 0.60 ± 0.17 (n = 3). The A_{280} peak at 2.5 min is an injection artifact with a constant height. In some cases a small amount of GTP-binding activity was observed in this peak. About 0.3 to 0.4 [^{35}S]GTPγS was bound per mole of protein (*13*). The actual binding is probably higher, since binding of many small proteins to nitrocellulose is less than 100% efficient. GTPase was measured at 30°C in 0.20 ml of 20 m*M* tris-HCl, *p*H 7.4, containing 1 m*M* EDTA, 1 m*M* dithiothreitol, 0.8*M* NaCl, and 0.25 μ*M* γ[^{32}P]GTP (10 to 30 Ci/mmol). After 30 min [^{32}P]Pi was reacted with phosphomolybdic-silicotungstic acid (*21*) and extracted into benzene. GTPase is expressed as disintegrations per minute of ^{32}P released per 30 min. [^{35}S]GTPγS binding was measured under identical conditions except that the *p*H was 8.0 and 3.4 n*M* [^{35}S]GTPγS (3.7 μmol/Ci) was added instead of GTP. After 2 hours, bound ^{35}S was measured after filtration on nitrocellulose (*11*). A blank of 200 dpm has been subtracted from the GTPase data.

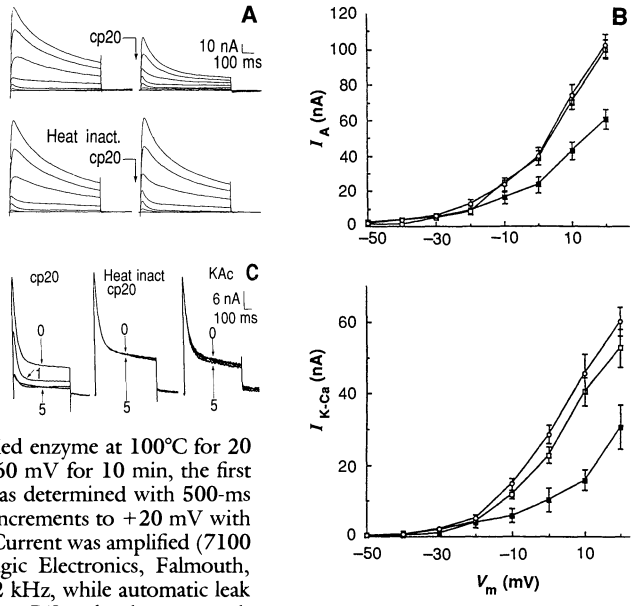

Fig. 4. Voltage-clamp recordings before and after iontophoretic injections of cp20. Cp20 was purified by ion-exchange HPLC followed by size-exclusion HPLC and concentrated as in Fig. 3, then partially lyophilized. Cp20 (0.09 μg) was redissolved in 10 μl of 1*M* KAc and injected into type B neurons submerged in artificial sea water (*p*H 7.4, 900 mOsm, containing 430 m*M* NaCl, 10 m*M* KCl, 10 m*M* $CaCl_2$, 50 m*M* $MgCl_2$, 10 m*M* tris-HCl). Heat-inactivated cp20 was prepared by incubation of purified enzyme at 100°C for 20 min. After cells were held at −60 mV for 10 min, the first current-voltage (*I-V*) relation was determined with 500-ms steps from −60 mV at 10-mV increments to +20 mV with one pulse occurring every 60 s. Current was amplified (7100 Voltage Clamp Amplifier, Pelagic Electronics, Falmouth, Massachusetts) and sampled at 2 kHz, while automatic leak subtraction was effected with a P/8 subpulse protocol. Immediately after determining the first *I-V* relation, −2.0 nA was applied through the voltage electrode for 2 min, then a second *I-V* relation was measured. (**A**) Effect of injections of cp20 or heat-inactivated cp20 on the early and late K^+ currents. The early K^+ current, reaching a maximum within 20 ms, was I_A (*22*), and the late K^+ current, reaching a maximum within 250 ms, was shown at potentials <0 mV to be mainly $I_{K\text{-}Ca}$ (*22*). Current records were digitally filtered (3 kHz) off-line. (**B**) *I-V* relations of (upper) I_A and (lower) $I_{K\text{-}Ca}$ currents 1 min after injection of cp20 or control solutions. Mean ± SEM; n = 4. (**C**) Time course of cp20 inhibition of K^+ currents. A minimum was reached 5 min after injection and the currents remained depressed at 15 min.

late K^+ channels, particularly $I_{K\text{-}ACh}$ (*10*). Several low molecular weight G proteins, such as N-ras p21, exist in mammalian and invertebrate cells (*16–18*) and can induce cellular growth or proliferation (*17, 19*). Thus, cp20 may belong to a class of proteins that cannot only regulate ion currents, but may also induce the structural changes that occur after conditioning in these neurons.

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8. The ^{32}P incorporation was calculated on the basis of the in vivo specific activity of $\gamma[^{32}P]$adenosine triphosphate (ATP) and calibration of the A_{280} peak areas with bovine serum albumin (BSA) (*13*). The in vivo specific activity of ATP, measured after a 3-hour incubation of isolated eyes with $[^{32}P]$Pi, by reversed-phase HPLC (5% tetrabutylammonium phosphate in 27% methanol in water), was 7.69 ± 10^{14} dpm/mol.
9. Since the rates of phosphorylation and dephosphorylation are much faster than the rate of protein synthesis in *Hermissenda* eye, over the 6-hour incubation period, most newly synthesized protein would be phosphorylated to the same extent as previously existing protein. Thus, the specific activity is largely a measure of synthesis of the protein molecule irrespective of its state of phosphorylation. No significant difference was detectable in the ^3H in the peaks with t_R's of 0 to 26 min, which contained the unincorporated amino acids, indicating that there were no differences in ^3H uptake among the groups. Previous results also indicated no differences in $[^{32}P]$Pi uptake (7).
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13. This value is based on a total ^{35}S binding of the peak in Fig. 3 (after correcting for decay, counting efficiency, and quenching by nitrocellulose) of 132,000 dpm, which is equivalent to 2.32×10^{-13} mol of GTPγS. This was divided by the amount of protein (peak area, 59,000 μV-sec = 1.18×10^{-8} g of protein = 5.9×10^{-13} mol of cp20) to give a value of 0.39 mol of GTPγS per mole of protein. The absorbance/gram of cp20 at 280 nm was 0.96 times that of BSA: Purified cp20 was passed through a Sephadex G-25-50 column and the area of the protein peak, at 210 and 280 nm, was compared with the areas obtained from injections of BSA. Cp20 gave 1.16 times as strong a signal per gram as BSA (using the A_{210} result as 1.00) in an Aurodye colorimetric protein assay [C. M. Stoscheck, *Anal. Biochem.* **160**, 301 (1987)], modified as follows: 0.85 ml of Aurodye was mixed with 0.05 ml of 1*M* citric acid (*p*H 3.0) plus 0.1 ml of sample and reacted for 45 min at 45°C, then A_{595}/A_{520} was measured. The three methods gave values within 15% of each other.
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