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Information storage in the nervous system of *Aplysia*: Specific proteins affected by serotonin and cAMP

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ABSTRACT **To identify proteins that may be involved in the induction of long-term changes in the nervous system, we investigated whether specific proteins in pleural sensory neurons of *Aplysia* were affected by procedures that mimic those used to produce long-term sensitization. Using two-dimensional PAGE, we found that exposure to serotonin (5-hydroxytryptamine, 5-HT) for 2 or 3 hr appeared to increase incorporation of labeled amino acids into one protein (P9) and decrease incorporation into two other proteins (P19 and P20). These effects of 5-HT were observed whether the labeled amino acid was leucine or methionine. The same proteins that were affected by 5-HT were also altered by the adenylate cyclase activator forskolin and by the 8-bromo and 8-benzylthio analogs of cAMP. Addition of Co^{2+} to 5-HT did not seem to affect the action of 5-HT on P9 and P20, but it did seem to block the effect of 5-HT on P19. However, the effect of analogs of cAMP on P9, P19, and P20 was not altered by inclusion of Co^{2+}. A phorbol ester that activates protein kinase C did not appear to affect the proteins that were modified by 5-HT, but phorbol ester did appear to increase the amount of labeled amino acids incorporated into another protein (P24). To investigate the specificity of these effects for pleural ganglion neurons, we examined the effect of 3- and 6-hr treatments of 5-HT on proteins in the abdominal ganglion. 5-HT affected at least nine proteins in the abdominal ganglion. One of these proteins (P9) appeared to be the same as one altered by 5-HT in the pleural sensory neurons. However, the occurrence of some proteins and some effects of 5-HT were specific for one ganglion or the other. The identified proteins that were affected by both 5-HT and changes in cAMP may be involved in the induction of long-term changes in the nervous system of *Aplysia*.**

A fundamental issue in the cellular analysis of memory is the relationship among the mechanisms underlying the induction, storage, and retrieval of short-term and long-term memory. Recently, this issue has begun to be investigated at the cellular and molecular levels in the marine mollusc *Aplysia*. Simple defensive reflexes in this animal exhibit both short-term (lasting minutes) and long-term (lasting days) sensitization (reviewed in refs. 1 and 2). Short-term sensitization is correlated with intrinsic changes in synaptic efficacy of the connections between primary sensory neurons and their follower interneurons and motor neurons (1–6). The induction of short-term sensitization is due, at least in part, to an elevation of the levels of cAMP in the sensory neurons as a result of sensitizing stimuli (7, 8). Altered levels of cAMP modulate specific membrane channels and perhaps other subcellular processes as well (9–15). The short-term effects of sensitizing stimuli can be mimicked by application of serotonin (5-hydroxytryptamine, 5-HT) or the peptide SCP_B (6, 16, 17). Interestingly, the sensory neurons are also a locus of long-term sensitization (18, 19), and long-term changes can also be induced by 5-HT and cAMP (5, 20–23). A critical difference, however, is that inhibitors of protein synthesis block some long-term changes produced by 5-HT and cAMP but do not affect short-term changes (21–23).

Although progress has been made in understanding the mechanisms of long-term sensitization, proteins involved in the induction of long-term sensitization remain to be discovered. We have begun to explore this issue by determining whether proteins in pleural sensory neurons are altered by procedures that mimic long-term behavioral training. Using two-dimensional PAGE, we found that 2 or 3 hr treatments with 5-HT or agents that elevate cAMP led to changes in incorporation of labeled amino acids into at least three proteins in pleural sensory neurons. A phorbol ester that activates protein kinase C did not appear to affect those proteins changed by 5-HT. In the abdominal ganglion, several additional proteins were also affected by 5-HT.

MATERIALS AND METHODS

Aplysia californica were kept in an aquarium containing artificial sea water (ASW; Instant Ocean, Eastlake, OH) at 15 ± 1°C. After at least a 2-day equilibration period, animals were anesthetized by injecting them with a volume of isotonic $MgCl_2$ equal to approximately one-half their body volume. A right and a left pleural ganglion were removed from six animals in each experiment. The pleural ganglia contain the sensory neurons that innervate the posterior portion of the animal and mediate the tail and tail-siphon withdrawal reflexes (19, 24, 25). One pleural ganglion from each animal was placed in the experimental treatment group, and the contralateral ganglion was placed in the control group. Three right and three left ganglia were used for each group.

Pleural Sensory Neurons. Groups of six pleural ganglia were pinned to the floor of a 3-ml chamber and allowed to equilibrate for 2 hr at 15 ± 1°C. The chambers were filled with a control sea water (CSW) solution of ASW (pH 7.65) containing streptomycin at 100 μg/ml, penicillin G at 1000 units/ml, and 30 mM Hepes. After the 2-hr equilibration period, one of two basic treatment procedures was utilized. In the first procedure, the two chambers were washed with a solution of CSW containing either L-[2,3,4,5-^{3}H]leucine (160 μCi/ml; 1 Ci = 37 GBq) or L-[^{35}S]methionine (150 μCi/ml). After 1 hr in the label, a concentrated volume of either serotonin (final concentration = 5 μM), forskolin (1 μM), 8-benzylthio-cAMP (1 mM), 8-bromo-cAMP (1 mM), or phorbol 12-myristate 13-acetate (PMA; 10 μM) was added to the experimental chambers for 2 hr. The second procedure was similar to the first except that the labeled amino acid and agonist were applied simultaneously for 3 hr. After the 2- or 3-hr treatment period, both chambers were drained and ASW with propylene glycol at −5°C was added to the ganglia.

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Abbreviations: 5-HT, serotonin (5-hydroxytryptamine); PMA, phorbol 12-myristate 13-acetate; ASW, artificial sea water; CSW, control sea water.

While still frozen, clusters of sensory neurons were removed and then placed into 0.1 ml of solubilization buffer {9.1 M urea/2% (wt/vol) ampholytes/5% (vol/vol) 2-mercaptoethanol/3% (wt/vol) 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS)}. After 2 hr at room temperature, the samples were frozen (−70°C).

Abdominal Ganglion. After excess tissue adherent to the sheath was trimmed from six ganglia, three abdominal ganglia were pinned to the floor of two separate 1-ml chambers. The ganglia were allowed to equilibrate in CSW at 15°C for 2–12 hr before treatment. In one experimental procedure, experimental ganglia were exposed to 5-HT (5 μM) for 6 hr and to [^{3}H]leucine (160 μCi/ml) during the last 4 hr of 5-HT treatment. Control ganglia were exposed only to 4 hr of [^{3}H]leucine. In a second experimental procedure, the treatment consisted of a simultaneous 3-hr exposure to 5-HT and [^{3}H]leucine (200 μCi/ml), whereas the control treatment consisted of a 3-hr exposure to [^{3}H]leucine only.

After treatments, ganglia were rinsed and then homogenized on ice in 50 μl of grinding buffer [50 mM Tris/5 mM EDTA/1 mM EGTA/bacitracin (0.125 mg/ml)/benzamidine (1.5 mg/ml)/soybean trypsin inhibitor (0.125 mg/ml)/aprotinin (0.8 trypsin inhibitor unit per ml)/phenylmethylsulfonyl fluoride (0.175 mg/ml)] and then further homogenized after addition of 50 μl of solubilization buffer. Urea was then added to the homogenate to make the final concentration of urea 8 M.

Electrophoresis. Equal amounts of radioactivity incorporated into protein (26) of experimental and control samples were loaded on separate gels. The proteins in samples were separated by O'Farrell's technique (27) for two-dimensional PAGE with a few changes. The size was reduced by using Minigels (Idea Scientific, Corvallis, OR) in the second dimension. A pH gradient of about 4–7 was established with a mixture of ampholytes (Pharmacia) such that the final concentration of ampholytes in the isoelectric focusing gel solution was 2% (wt/vol). Isoelectric points of specific proteins were estimated by using markers obtained from Sigma: carbonic anhydrase B from human erythrocytes (pI 6.6), carbonic anhydrase B from bovine erythrocytes (pI 5.8), β-lactoglobulin A from bovine milk (pI 5.1), and soybean trypsin inhibitor (pI 4.6). Molecular weight standards, pI marker proteins, and sample proteins were located on the slab gels by a silver staining procedure (Gelcode; Health Products, Rockford, IL).

Other details of the gel techniques have been previously published (26, 28). Experimental samples and their matched controls were run on the same batch of isoelectric focusing tube and slab gels. Replica two-dimensional gels were done for each experimental and control sample. One experimental and one control gel were dried on the same piece of filter paper and then placed under a single sheet of Kodak XAR film. Many gels were exposed to film for different durations of time (1–4 weeks) to ensure that the film was not saturated for certain protein spots. Each pair of experimental and control gels was analyzed independently.

Gels were analyzed visually and, in some cases, with an automated densitometer from Technology Resources (Nashville, TN). For a given treatment, usually three individuals visually inspected gels for effects. Once potentially interesting proteins were identified, three individuals scored the proteins on each autoradiogram for increases, decreases, no effects, or data not available. Large inconsistencies between individual scorers were examined and resolved. We used two general criteria for defining an effect of a treatment. First, the effect had to be reproducible in the replica gels of a given experiment. Second, the effect had to be reproducible over three or more independently performed experiments.

The automated densitometer was utilized to obtain the summed optical densities over the area of the protein spot. Then the ratio of summed optical densities of an experimental to a control protein spot was obtained with the gel comparison program. This ratio was corrected for differences in label of the gels by normalizing the summed optical density values for each spot of interest with the total summed optical densities of all protein spots on the autoradiograms. The result for each spot in an individual experiment was the geometric mean of the ratios obtained from the replica experimental–control gel pairs. The overall effect of a treatment was computed as the geometric mean of the ratio values for each individual experiment. Geometric means of the data were used because they are more representative of samples composed of ratios (e.g., experimental/control densities) than are the arithmetic means (29). The ratios obtained from the computer gel analysis only approximate the magnitude of experimental changes in incorporation of label because of the nonlinear relationship between the summed optical density and the amount of label associated with a protein in the gel. Autoradiograms were not calibrated to define this relationship.

RESULTS

5-HT Changes Several Proteins in Pleural Sensory Neurons. Long-term sensitization of the tail-siphon reflex is observed following a 1.5-hr training session (19). 5-HT is a candidate transmitter that mediates the effects of sensitizing stimuli (2, 6, 11, 17, 30). To roughly mimic sensitization training and to maximize our chances for detecting changes in incorporation of label into specific proteins, we exposed pleural ganglia to 5-HT (5 μM) for 2 or 3 hr. 5-HT produced reproducible changes in incorporation of label into at least three proteins found in sensory neurons of pleural ganglia as indicated by the arrows on Fig. 1 and as summarized in Table 1. One prominent effect of 5-HT was increased incorporation of label into an ≈43-kDa, 5.3 pI protein that we refer to as P9. Two other changes involved 5-HT-induced decreases in incorporation of label into an ≈43-kDa, 5.3 pI protein that we refer to as P19 and a 46-kDa, 5.2 pI protein that we refer to as P20. Similar effects were observed in two experiments in which [^{3}H]leucine was used and in seven experiments in which [^{35}S]methionine was used. Also, the effects were similar when either 2- or 3-hr 5-HT treatments were used.

Densitometric analysis of the gels yielded results that were consistent with those produced by visual analysis. The mean ratio (experimental/control) of the summed optical density values for 2-hr 5-HT treatments for P9 was 1.52, $n = 7$ (values for each experiment: 1.24, 1.31, 1.01, 1.29, 2.14, 2.07, 2.01) and for P19 was 0.86, $n = 7$ (0.80, 0.87, 0.80, 0.89, 0.65, 1.15, 0.97). Ratios for P20 could not be reliably obtained because a P20 spot was not observed on most of the experimental gels. Two other proteins (P1 = 78 kDa, pI 5.6; P3 = 68 kDa, pI 5.5) that showed somewhat variable effects appeared potentially interesting as a result of visual analysis. Computer analysis indicated that 5-HT did not affect P1 and P3 [P1: mean ratio (experimental/control) = 1.28; values: 2.25, 1.80, 0.91, 1.02, 0.82, 1.44; P3: mean ratio = 0.98; values: 1.85, 1.06, 1.63, 1.01, 0.47, 0.87, 0.65]. Though the mean of the ratio for the P1 protein is larger than 1.00, the variation in the results of individual experiments indicated 5-HT treatment had no consistent effect on P1.

The reciprocal effects of 5-HT on P9 (an increase) and P19 (a decrease) together with their similar molecular masses and pI values suggest a relationship between the proteins and the two effects of 5-HT. For example, a treatment that modifies the charge of a protein or adds charge (e.g., phosphorylation) would shift some "density" along the pI dimension of the autoradiogram. If the effect of 5-HT were due to such a mechanism, one would predict that the magnitude of changes in P9 would be negatively correlated with changes in P19. We

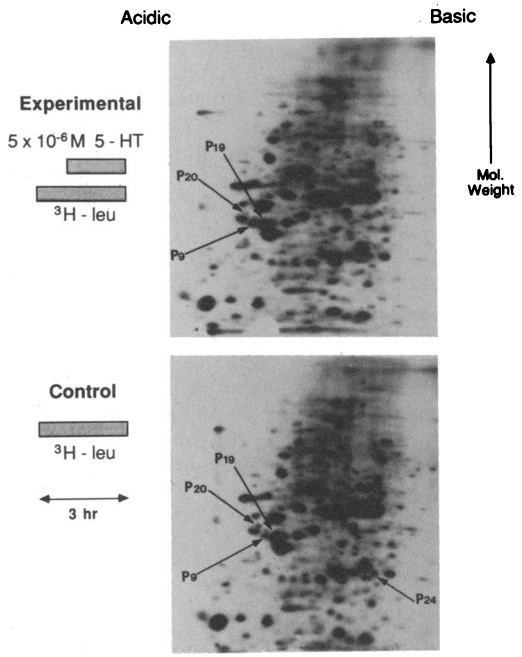

FIG. 1. Effect of 5-HT on the incorporation of [^{3}H]leucine (^{3}H-leu) into proteins detected by autoradiography of two-dimensional gels. (*Upper*) An experimental group of isolated pleural ganglia were exposed to [^{3}H]leucine for 3 hr and 5-HT (5×10^{-6} M) during the last 2 hr of exposure to label. (*Lower*) Control contralateral pleural ganglia were only exposed to [^{3}H]leucine for 3 hr. After treatments, clusters of sensory cells were removed from pleural ganglia and electrophoresis was performed on samples prepared from these clusters of sensory neurons. P24 was not affected by treatment with 5-HT but was affected by treatment with PMA.

examined this prediction by graphing P9 vs. P19 values of each gel pair for all of the 2-hr experiments with 5-HT. From the graph, P9 and P19 exhibited a small positive correlation ($r = 0.22$) instead of the predicted negative correlation. This finding suggests that the observed effects of 5-HT on P9 and P19 are independent (see below also).

Effects of 5-HT Are Mimicked by Elevating cAMP but Not by Activating Protein Kinase C. If the above changes in incorporation of label produced by 5-HT are related to the induction of sensitization, one would predict that the effects of 5-HT could be mimicked by changes in cAMP. To test this prediction, ganglia were treated for 3 hr with forskolin (an activator of adenylate cyclase) or the 8-bromo (three experiments with [^{35}S]methionine) or 8-benzylthio (two experiments with [^{3}H]leucine) analogs of cAMP. In eight of eight experiments in which forskolin was used, the change in P9 was similar to that produced by 5-HT. In only four of these eight experiments was it possible to identify P20. In three of these four experiments, the incorporation of label into P20 was decreased. Data for P19 was not available from most of these forskolin experiments because of insufficient separation of P19 from a protein of higher molecular mass. In five of five experiments in which the cAMP analogs were used, an increase in incorporation of label was observed in the P9 protein (Fig. 2). In four of the five experiments using cAMP analogs, clear decreases in incorporation of label in the P19 and P20 proteins were observed. These results, summarized in Table 1, indicate that 5-HT and treatments that increase cAMP produced similar effects on P9, P19, and P20.

We also examined the effects of activation of protein kinase C, since some recent results indicate that protein kinase C may have a role in short-term facilitation in *Aplysia* (31, 32). PMA, a phorbol ester known to activate protein kinase C in *Aplysia* (32, 33), appeared to produce no consistent change in P9, P19, or P20 (four experiments). However, PMA did appear to increase incorporation into a protein (P24) with an approximate molecular mass of 36 kDa and pI of 5.8 (computer analysis mean = 1.53, individual values: 1.13, 1.49, 2.57, and 1.27). The location of P24 is indicated on Figs. 1 and 2.

Effect of Co^{2+} on Responses to 5-HT and Analogs of cAMP. Because intact ganglia were exposed to 5-HT, 5-HT may have acted indirectly by causing release of modulatory transmitters from other neurons, which in turn affected the sensory neurons. To examine this possibility, we repeated the above experiments with 5-HT in the presence of CSW containing 30 mM Co^{2+} (Co^{2+}/CSW). This concentration of Co^{2+} is known to block Ca^{2+} influx and transmitter release in *Aplysia* (34, 35). Control groups of ganglia were exposed to Co^{2+}/CSW. In nine out of nine experiments, increases in incorporation of label into P9 were still observed. In five of these nine experiments, decreases in incorporation of P20 were also observed. P20 could not be detected in the experimental or control gels of three of the other nine experiments. These results indicate that changes in the P9 and P20 proteins were due to a direct action of 5-HT on serotonin-sensitive receptors of sensory neurons. However,

Table 1. Effects of treatments on proteins

Parameter	Protein											
	P9	P19	P20	P24	A4	A5	A10	A11	A12	A70	A120	A130
Molecular mass, kDa	43	43	46	36	63	39	35	32	55	27	74	26
pI	5.3	5.3	5.2	5.8	5.4	5.6	5.3	5.3	6.4	5.5	5.1	5.8
Present in pleural	Yes	Yes	Yes	Yes	Yes	No	Yes	No	Yes	Yes	No	Yes
Present in abdominal	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Pleural treatment*												
5-HT	+	−	−	0	0		0		?	0		0
5-HT + Co^{2+}	+	0	−	0								
Forskolin	+	?	−	0								
cAMP analogs	+	−	−	0								
cAMP analogs + Co^{2+}	+	−	−	0								
PMA	0	0	0	+								
Abdominal treatment†												
5-HT (3 hr)	+	0		?	0	0	+	0	−	−	+	−
5-HT (6 hr)	+	?			−	+	+	+	−	?	0	?

*Pleural treatment refers to experiments in which the treatments (2–3 hr) were administered to isolated pleural ganglia and gels were run on clusters of sensory cells that were removed from the ganglia. +, Increased incorporation of label; −, decreased incorporation of label; 0, no change in incorporation of label; ?, more information is required.

†Abdominal treatment refers to experiments in which isolated ganglia received experimental treatments and gels were run on samples of homogenized ganglia.

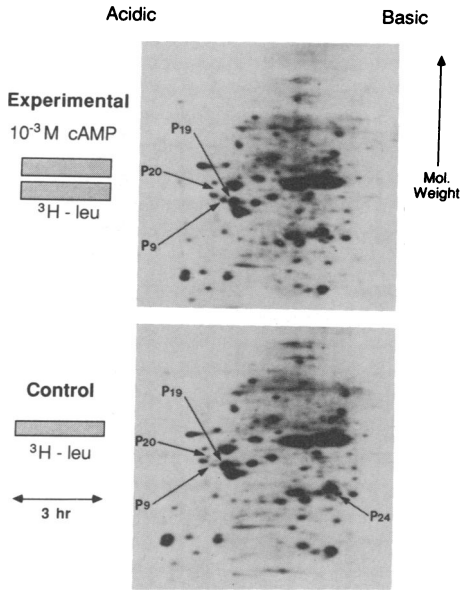

FIG. 2. Effect of 3-hr treatment of 8-benzylthio-cAMP (10^{-3} M) on incorporation of label into proteins of pleural sensory neurons. (*Upper*) Ganglia were treated for 3 hr with 8-benzylthio-cAMP. [^{3}H]Leucine was also present during this incubation. (*Lower*) Ganglia were only exposed to [^{3}H]leucine for 3 hr.

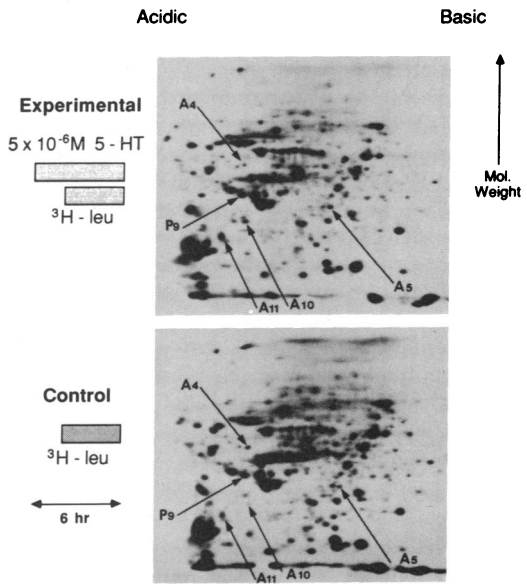

FIG. 3. Effect of a 6-hr treatment of 5-HT on incorporation of label into proteins of abdominal ganglia. (*Upper*) Abdominal ganglia were exposed to 5-HT (5×10^{-6} M) for 6 hr. [^{3}H]Leucine was also present for the last 4 hr. (*Lower*) Ganglia were only exposed to [^{3}H]leucine for 4 hr. At least one protein (e.g., P9) affected by 5-HT in the pleural sensory cells also seemed to be affected by treatment of the abdominal ganglion with 5-HT. Some additional proteins were affected by 5-HT in the abdominal ganglion. A few examples of the additional proteins in the abdominal ganglia are shown. The abundance of label associated with A5, A10, and A11 increased, whereas that associated with A4 decreased as a result of 5-HT treatment.

Co^{2+} appeared to block the effect of 5-HT on P19. In six of nine experiments, P19 did not appear to be affected by the Co^{2+}/CSW treatments. In the other three experiments with Co^{2+}/CSW, P19 appeared increased in one experiment and decreased in the other two experiments. This differential effect of Co^{2+} on the ability of 5-HT to affect P9 and P19 further indicates that the effects of 5-HT on P9 and P19 are not related to one another and that the two proteins are different.

The above results indicate that analogs of cAMP mimic the effects of 5-HT on proteins and that a Ca^{2+}-requiring process may be required in the effect of 5-HT on P19. To further study the action of Co^{2+}, we investigated, in six experiments, the effects of 8-benzylthio-cAMP plus Co^{2+} treatments on pleural ganglia. As a result of 8-benzylthio-cAMP plus Co^{2+} treatments, the label associated with P9 was increased and that associated with P19 and P20 was decreased.

Effects of 5-HT on the Intact Abdominal Ganglion. Since the effects of 5-HT were limited to a few proteins and because we were interested in examining the specificity of the effects of 5-HT on pleural sensory neurons, we investigated the effects of 5-HT on proteins in the abdominal ganglion. In these experiments, we used both 3-hr (nine experiments) and 6-hr (four experiments) exposures to 5-HT (see Table 1). Both 3- and 6-hr treatments of 5-HT led to enhanced incorporation of label into a protein that appeared at the same location on autoradiograms as P9 (Fig. 3). A protein in the abdominal ganglion with a similar molecular mass and pI to P19 did not appear to be affected by 5-HT. We could not detect a protein similar to P20 in the abdominal ganglion. Eight additional proteins affected by 5-HT were discovered in the abdominal ganglion (Table 1). Several changes were observed in proteins of the abdominal ganglion that were not exhibited by similar-appearing proteins of the pleural sensory neurons. For example, both 3- and 6-hr exposures to 5-HT led to increased incorporation into an ≈35-kDa, pI 5.3 protein (A10), and 3-hr exposures to 5-HT led to decreased incorporation into ≈27-kDa, 5.5 pI (A70) and 26-kDa, 5.8 pI (A130) proteins (Table 1). In addition, 6-hr exposures to 5-HT led to increased incorporation of label into an ≈39-kDa, 5.6 pI protein (A5), and 3-hr exposures to 5-HT led to enhanced incorporation of label into an ≈74-kDa, 5.1 pI protein (A120). Proteins like A5 and A120 were not found in autoradiograms of pleural ganglia cells. Also, there were a few proteins (A4, A5, and A11) found in the abdominal ganglia that appeared to be affected by 6-hr 5-HT treatments but not by 3-hr 5-HT treatments, and there was a protein (A120) that appeared to be affected by 3-hr 5-HT treatments but not by 6-hr 5-HT treatments.

DISCUSSION

Our results indicate that 2- to 6-hr treatments of 5-HT produced changes in incorporation of labeled amino acids into specific proteins in pleural sensory neurons and abdominal ganglia (Table 1). 5-HT appeared to increase the label associated with some proteins (P9, A5, A10, A11, and A120) and decrease label associated with others (P19, P20, A4, A12, A70, and A130). The effects of 5-HT on proteins of pleural ganglia cells were evident whether labeled leucine or methionine was used. Our results obtained with 5-HT plus Co^{2+} treatments suggest that the effects of 5-HT and analogs of cAMP on P9 and P20 were produced by a direct action of 5-HT upon sensory neurons. Previously, the ability of 5-HT to elevate cAMP in pleural sensory neurons was demonstrated (16). The effects of 5-HT on P9, P19, and P20 proteins of pleural sensory neurons appeared to be mediated by cAMP. PMA did not appear to change the proteins in the pleural ganglia that were affected by 5-HT, but PMA appeared to increase incorporation of label into P24.

Too little is known about the proteins that we identified to discuss their cellular functions. However, the properties of P19 look interesting. P19 was affected by both 5-HT and analogs of cAMP. The effect of 5-HT on P19 appeared to be blocked by Co^{2+}, but the effect of an analog of cAMP on P19 was not blocked by Co^{2+}. These results indicate that an additional agonist is involved in the effect of 5-HT on P19 or

that a flux of Ca^{2+} into the cell is required for the effect of 5-HT on P19. The possibility that both cAMP and Ca^{2+} are mediating the effect of 5-HT upon P19 is intriguing.

Comparisons of the distribution of the proteins and the effects of 5-HT on the proteins between the pleural and abdominal ganglia were interesting (Table 1). Eight of the proteins (P9, P19, P24, A4, A10, A12, A70, and A130) that we identified were observed in autoradiograms from both ganglia, whereas four of the proteins (P20, A5, A11, and A120) were only seen in autoradiograms from one or the other ganglion. Differences in proteins may relate to different functions of cells in the pleural and abdominal ganglion and/or different types of cells in the two preparations. In our studies of abdominal ganglia, gels were run on samples of homogenized whole ganglia, whereas in studies of pleural ganglia, gels were run on samples of isolated clusters of sensory cells. Thus, the presence of A5, A11, and A120, which were found only in preparations of the abdominal ganglion, may reflect a nonneuronal origin of these proteins or it may reflect different populations of neurons that subserve the different functions of the abdominal and pleural ganglia. Also, our findings that proteins P19, A4, A10, A70, and A130 were affected by 5-HT in one of the ganglia but not the other indicate that the same protein may be regulated in different ways in different cells.

The changes we observed in incorporation of label into protein probably do not include all of the changes that were induced. Gel techniques are selective and it is likely that different proteins will be affected depending upon the duration of treatment and the time at which proteins are analyzed after treatment. Indeed, proteins A4, A5, and A11 in the abdominal ganglion appeared to be affected by 6-hr but not 3-hr exposures to 5-HT. Also, Castellucci *et al.* (5, 36) have identified changes in incorporation of labeled amino acids into proteins in sensory neurons of abdominal ganglia 24 hr after sensitization training. These proteins appear to be different from the ones described in our report.

Our results do not tell us how the observed changes of proteins were produced. One obvious possibility is that 5-HT changed uptake of amino acids. This seems unlikely because the ratio of trichloroacetic acid-precipitable counts for experimental/control was 1.01 for 5-HT (10 experiments) and 0.96 for forskolin (11 experiments). In addition, incorporation of label into two proteins was decreased (P19 and P20), while incorporation into another protein was increased (P9). Another possible mechanism for producing differences in incorporation involves stimulation of peptide synthesis associated with the release of peptides. Release of egg-laying hormone and treatments that increase cAMP levels stimulate neuropeptide synthesis in bag cells of *Aplysia* (37, 38). This mechanism does not appear to be involved in at least some of the changes we observed because the effects of 5-HT on pleural ganglion proteins were present in conditions when peptide release should have been inhibited by Co^{2+}.

Irrespective of the mechanism of the observed changes, the proteins we have identified in pleural and abdominal ganglia may play important roles in the plasticity of *Aplysia* neurons induced by 5-HT, cAMP, or activation of protein kinase C. Thus far, our results have focused attention upon a number of proteins worthy of future study. Elucidation of the mechanisms of change as well as the cellular functions of the identified proteins will be required to determine if these proteins play a role in the molecular storage of environmental information.

Note Added in Proof. A preliminary report of our results has been presented (39), and Barzilai *et al.* (40) also reported that 5-HT changed incorporation of labeled amino acids into proteins of pleural sensory neurons.

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