MEMBRANE RESPONSES AND CHANGES IN cAMP LEVELS IN APLYSIA SENSORY NEURONS PRODUCED BY SEROTONIN, TRYP TAMINE, FMRFamide AND SMALL CARDIOACTIVE PEPTIDE$_B$ (SCP$_B$)

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While recent evidence indicates a role for serotonin (5-HT) in modulating the defensive tail-withdrawal reflex in Aplysia, little information exists concerning the specificity of these 5-HT effects. As a first-step in addressing this issue we have examined the dose-response relationship for one aspect of the 5-HT modulation (enhancement of cAMP levels in isolated clusters of sensory neurons) and compared the effects of 5-HT with three potential neurotransmitters: tryptamine, FMRFamide (Phe-Met-Arg-Phe-NH$_2$) and small cardioactive peptide$_B$ (SCP$_B$). Cyclic adenosine monophosphate (cAMP) levels were enhanced as a graded function of the concentration of 5-HT with an EC$_{50}$ of 14 $\mu$M. At a concentration of 5 $\mu$M, both 5-HT and SCP$_B$ produced nearly identical increases in the cAMP content of sensory neurons. In contrast, 5 $\mu$M tryptamine or 5 $\mu$M FMRFamide had little or no effect on cAMP levels. We also examined the effects of these agents on membrane currents and membrane conductance. Both 5-HT and SCP$_B$ produced an inward current associated with a decrease in input conductance. Tryptamine had little or no effect, while FMRFamide produced a response opposite to that of 5-HT and SCP$_B$; an outward current associated with an increase in membrane conductance.

The connections between sensory neurons and motor neurons mediating the defensive tail-withdrawal reflex of Aplysia exhibit several plastic properties including heterosynaptic facilitation and activity-dependent neuromodulation [24, 26]. The heterosynaptic facilitation is mimicked by bath application of serotonin (5-HT) [26] and this transmitter acts by producing spike broadening and decreases in membrane K$^+$ conductance in the sensory neurons, which is mediated by increases in intracellular cAMP levels [4, 16–18, 21–23, 26]. Despite the evidence indicating a neuromodulatory role for 5-HT in tail withdrawal there is a paucity of information concerning the specificity of the pharmacological effects.

As a first-step in addressing this issue we investigated the dose-response relationship of 5-HT to increases in the cAMP levels of tail sensory neurons. In addition, we compared the ability of 5-HT to enhance cAMP levels with that of three other
agents: a structurally related compound, tryptamine, and two neuroactive peptides, a small cardioactive peptide (SCP\(_B\)) and FMRFamid.

For these experiments, animals were anesthetized with isotonic MgCl\(_2\) and the paired, pleural ganglia from each animal were removed and desheathed. A homogeneous cluster of sensory neurons, containing the tail sensory cells, was isolated from each pleural ganglion (for details see ref. 17). Isolated clusters were exposed to concentrations of 5-HT ranging from \(5 \times 10^{-7}\) M to \(10^{-3}\) M (experimental treatment) or artificial sea water (ASW; control treatment) for a total of 5 min. Both the experimental and control groups had equal numbers of clusters from the right and left ganglia. The phosphodiesterase inhibitor Ro 20-1724 was included in all solutions at a concentration of \(10^{-4}\) M. Immediately following exposure to 5-HT or ASW, clusters were frozen in 10% trichloroacetic acid, and their cAMP content was determined by radioimmunoassay and normalized to protein content as described previously [2, 17]. Since each animal had two clusters of sensory neurons, one cluster always served as a control. The effect of the experimental treatment was expressed as a percent increase relative to the control. The dose-response relationship for 5-HT-stimulated increases in cAMP levels is shown in Fig. 1. The effective concentration for a half-maximal increase in cAMP content is approximately 14

![Fig. 1 (left). Dose-response relationship of 5-HT to elevated cAMP levels. The effect of 5-HT on the cAMP content of sensory neuron clusters is expressed as a percent of the cAMP content of the contralateral cluster receiving the control treatment. Each point on the graph represents the average percent increase determined for 12-13 animals. Average basal cAMP levels were 56.3 ± 2.7 pmol/mg protein, \(n = 86\). A non-linear parameter estimation program (based on the Gauss-Newton algorithm) was employed to generate the curve fit using a form of the Michaelis-Menten equation. The estimated EC\(_{50}\) value is 14 μM.](image)

![Fig. 2 (right). Comparison of the effects of 5-HT (Sigma), tryptamine (Sigma), SCP\(_B\) (Peninsula) and FMRFamide (Cambridge Research Biochemicals) on the cAMP content of sensory neuron clusters. Isolated clusters of sensory neurons were exposed for 5 min to 5 μM concentrations of 5-HT, tryptamine, SCP\(_B\) or FMRFamide. The effect on the cAMP content was determined as described in Fig. 1 (and see text). Cyclic cAMP levels were significantly elevated in response to treatment with 5-HT (\(t_{11} = 2.64, P < 0.05\), one-tailed \(t\)-test for non-independent groups). Similar significant elevations were produced by treatment with SCP\(_B\) (\(t_{12} = 2.0, P < 0.05\)). Tryptamine had only a nominal effect on cAMP levels and this effect was not significant (\(t_{12} = 0.23\)). FMRFamide also had no significant effect on the cAMP content (\(t_{13} = 0.65\)). Error bars indicate S.E.M.](image)
μM. These results are similar to those obtained on the abdominal ganglion [6], buccal muscle [13], heart [15, 20] and eye [9] of *Aplysia* (see also ref. 8).

We also compared the effect of 5-HT on the cAMP levels of sensory neuron clusters with that of tryptamine, FMRFamide and SCP₈. Tryptamine, like 5-HT, is an indolealkyl amine with the sole difference being the absence of a single hydroxyl group. Recently, SCP₈ [11, 12] has been isolated from *Aplysia* muscle and neural tissue [13] and has been shown to mimic the facilitatory effects of 5-HT on sensory neurons involved in gill and siphon withdrawal [5, 14]. In addition, several studies indicate that SCP₈ may elevate cAMP levels in the radula closer muscle [13] and in the abdominal ganglion [5]. FMRFamide [19] has been identified immunohistochemically in *Aplysia* gill [27] and CNS [3]. In the gill, FMRFamide evokes phasic contractions and augments cAMP levels [27], and facilitatory effects of FMRFamide on gill withdrawal have been reported [14].

Despite the structural similarity, a 5-min exposure to 5 μM tryptamine had only a minimal effect on the cAMP levels of sensory neurons compared to that produced by an identical exposure to 5-HT (Fig. 2). Treatment of sensory neuron clusters with 5 μM SCP₈ for 5 min resulted in an elevation of cAMP levels that was very similar to that seen in response to exposure to 5 μM 5-HT. However, a 5-min treatment with 5 μM FMRFamide had no discernable effect on the cAMP levels.

Previously, 5-HT was found to produce a cAMP-mediated inward current associated with a decrease in a resting K⁺ conductance in the tail sensory neurons [18, 21–23]. Since 5-HT and SCP₈ produced similar increases in cAMP levels, while application of FMRFamide and tryptamine produced little or no changes in cAMP levels, we were interested in examining the effects of these agents on the membrane current and input conductance of sensory neurons. For these experiments, individual sensory neurons were voltage-clamped at their resting potential (−40 to −50 mV) while 5 μl of concentrated stock solutions (10 mM) of the various agents were added to the experimental chamber, yielding a final concentration of 10 μM (Ro 20-1724 was not included). As for the biochemical experiments, surgically isolated clusters of sensory neurons were utilized in the electrophysiological experiments; however, slightly more neuronal tissue was included in the dissection to facilitate pinning the cells to the experimental chamber. In each experiment we first applied 5-HT and observed the characteristic inward current associated with a decrease in input conductance [23]. After a 5-min wash, one of the other agents was applied in a similar fashion. Using these procedures, we examined the effects of each agent in at least 6 different experiments and in multiple sensory neurons during each experiment. Fig. 3 illustrates one experiment where it was possible to test the effects of each agent on the same cell. The effects of SCP₈ typically were very similar to those of 5-HT and were associated with an inward current and decrease in input conductance. In contrast, tryptamine produced a small inward current, which in some cases was indistinguishable from the small current fluctuations produced by application of the vehicle alone (5 μl distilled water). In contrast to the inward currents and
Fig. 3. Transmitter effects on membrane conductance and current in tail sensory neurons. A sensory neuron in the left pleural ganglion was voltage-clamped at a holding potential of –40 mV with a single electrode voltage-clamp amplifier (Dagan 8100), while 5-mV constant-voltage hyperpolarizing pulses were applied to monitor changes in input conductance [23]. Bath application of 10 μM 5-HT (A) and SCPB (C) produced characteristic inward currents associated with decreases in input conductance. In this experiment 10 μM tryptamine (B) also produced an inward current associated with a decrease in input conductance, but the effects were considerably smaller than those of 5-HT and SCPB. The small effect of tryptamine was not due to lack of responsiveness of the cell, since 5-HT application after the tryptamine application produced a response comparable to that produced by the initial (A) 5-HT application (data not shown). FMRFamide (D) at a concentration of 10 μM produced a characteristic outward current associated with an increase in input conductance. The current displayed was the sampled current output of the voltage-clamp amplifier. The traces were aligned to correspond roughly to the start of the response.

decreases in membrane conductances produced by 5-HT and SCPB, FMRFamide consistently produced an outward current associated with an increase in input conductance. Similar outward currents produced by FMRFamide have been observed in *Helix* neurons [7].

The ability to generate a characteristic dose-response curve for the 5-HT-induced increase in cAMP levels, coupled with the inability of a closely related compound (tryptamine) to elevate cAMP levels, argues in favor of a specific, receptor-mediated action of 5-HT (see also ref. 1). The parallel between the biochemical effects of 5-HT, tryptamine, SCPB and FMRFamide and the electrophysiological effects of these agents is notable. Other attempts have been made, using the siphon sensory neurons of *Aplysia*, to correlate the electrophysiological and biochemical effects of 5-HT with more physiological stimuli [1]. However, confirmation of a physiological action of 5-HT must await the demonstration of specific serotonergic inputs to these sensory neurons.

It is interesting that, as has been observed for muscle and neural tissue in *Aplysia*, SCPB mimics the effects of 5-HT both in its ability to elevate cAMP levels [5, 13] and in its ability to produce heterosynaptic facilitation [5, 14]. This does not appear
to be a generalized effect of peptides on tail sensory neurons since FMRFamide has no effect on basal cAMP levels and exerts an opposite electrophysiological effect. The SCPB effects may be physiological since SCPB immunoreactivity has been observed for a small number of neuronal cell bodies in the pleural and pedal ganglia as well as for a large number of fibers which traverse these ganglia [10]. Evidence from the studies of Lloyd et al. [13] indicates that the 5-HT- and SCPB-induced increases in the cAMP content of Aplysia buccal muscle are mediated by separate receptors. They suggested the presence of parallel aminergic and peptidergic pathways which have similar actions. Such parallel pathways may also impinge on the tail-withdrawal circuit.

If physiological, the FMRFamide-induced outward current may have important consequences. It was previously demonstrated that a classical conditioning procedure involving the pairing of spike activity (conditioned stimulus; CS) in individual tail sensory neurons with the modulatory effects of tail shock (unconditioned stimulus; US) produced a temporally specific enhancement of the synaptic connections between the sensory and motor neurons involved in tail withdrawal [24]. At the subcellular level this enhancement, termed activity-dependent neuromodulation, is postulated to be the result of a synergistic effect of a Ca\(^{2+}\) influx during the CS and a component of the cAMP cascade (which is activated by the US) [9a, 16, 17, 24] Of particular interest is the observation that presentation of the US alone, or explicitly unpaired presentations of the CS and US, leads to a hyperpolarization of the sensory neurons [25] which is mediated, at least in part, by identified interneurons in the pleural ganglion (unpublished observations). It was suggested that the hyperpolarization may be important for endowing the system with temporal specificity since the hyperpolarization would tend to reduce a resting Ca\(^{2+}\) influx [25]. We are currently examining the possibility that FMRFamide may be the natural transmitter that mediates this inhibition by acting at the biochemical and membrane level to oppose the effects of the excitatory modulator(s).

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