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EXPRESSION OF SENSORIN, THE SENSORY CELL NEUROPEPTIDE, IS MODULATED BY SEROTONIN AND FMRF-AMIDE IN SENSORIMOTOR SYNAPSES OF *APLYSIA*.
L. Santarelli, S. Schacher # and P. G. Montarolo

Identified *Aplysia* neurons maintained in dissociated cell culture provide an excellent model system for studying the cellular and molecular changes accompanying long-term modulation of chemical connections. Previous studies indicated that serotonin (5-HT) and the peptide FMRF-amide are able to evoke respectively long-term facilitation or depression of sensorimotor connections that are accompanied by either an increase or a decrease in the number of presynaptic varicosities contacting the initial segment of the motor axons. These varicosities typically contain active zones for transmitter release. To examine whether the pre-existing varicosities as well as those present after each treatment express other synapse-specific markers, we examined the level of expression of sensorin- the neuropeptide specific for sensory cells in *Aplysia* (Brunet et al., 1991)- presynaptic varicosities following control treatments, 5-HT or FMRF-amide applications. We measured a) changes in the amplitude of the EPSP with intracellular recording, b) changes in the number of sensory varicosities by imaging dye fills of each sensory cell before and after each treatment, and c) the expression of sensorin by detecting the distribution of immunocytochemical staining of the cells after fixation with standard methods. As previously reported, 5-HT (N = 9 cocultures) and FMRF-amide (N = 9 cocultures) evoked functional and structural changes in the sensorimotor connections established after 5 days in culture compared to control treatment (N = 9 cultures). There was a significant variation in the amplitude of the EPSP and in the number of varicosities. As predicted from earlier morphological studies, the level of expression of sensorin was higher in the sensory varicosities contacting the proximal motor axons. Whereas 54%± 4.3 of the varicosities contacting the proximal axons were sensorin positive, only 22%± 3.5 of the varicosities contacting distal neurites or the substrate were sensorin positive. Following treatment with 5-HT, 73%± 6.3 of the pre-existing varicosities and 59%± 5 of the new varicosities were sensorin positive. In contrast, the number of sensorin positive varicosities following the application of FMRF-amide was reduced to a value of 34%± 3. These results suggest that 5-HT and FMRF-amide may modulate the expression of the sensory cell peptide either by modulating its synthesis or its turnover. In addition, changes in the properties of pre-existing varicosities may also contribute to both long-term facilitation and depression of the connection. Since many of the actions of 5-HT and FMRF-amide are produced respectively by cAMP and arachidonic acid dependent processes, it will be important to determine whether the change in sensorin expression is mediated by the same or other second messenger cascades.

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IS NITRIC OXIDE (NO) INVOLVED IN THE TRANSMISSION OF ACOUSTIC INPUT TO THE RAT AUDITORY CORTEX?

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NO has been proposed to act as an intercellular messenger, possibly involved in neuronal plasticity and in the transmission of somatosensory information. Aim of the present study is to check whether, at the inferior colliculus (IC) level, NO may participate in the processing and transmission of acoustic signals to the auditory cortex of the rat. Animals were anaesthetised with ketamine and xylazine (66 and 13 mg/kg, respectively, i.p.) and implanted with a stainless steel guide cannula to allow drug injection into the IC (0.5-1.0 µl at 1.0 µl/min rate). Auditory middle latency (MLR) and brain stem (ABR) responses were evoked by click stimuli of supramaximal intensity at 4/s and recorded from the auditory cortex by Ag/AgCl electrodes. IC injection of N^o-nitro-L-arginine methyl ester (L-NAME, 1.0 mM), an inhibitor of NO synthase (NOS), reduced by 51.7±6.6% (n=5) the amplitude of P₁₂-N₁ component of MLRs without significantly affecting the latency of MLRs as well as ABRs. This inhibitory effect was concentration-dependent and it ranged from 5.5±3.2% for the concentration of 0.5 mM (n=3) to 69.0±3.3% for 5.0 mM (n=5). L-NAME injection performed 10 min after pretreatment with the endogenous precursor of NO, L-arginine (5.0 mM), produced 24.2±3.6% reduction of P₁₂-N₁ amplitude and this was significantly lower (P<0.01) than that elicited by L-NAME given alone. IC injection of D-NAME (1.0 mM; n=5), the less active isomer of NAME, yielded no significant decrease in P₁₂-N₁ amplitude. IC infusion of dizocilpine (MK801; 1.0 µM, n=5) or LY274614 (1.0 mM; n=3), two selective NMDA receptor antagonists, reduced P₁₂-N₁ amplitude by 48.2±7.4% and 83.7±5.0%, respectively. The stereospecific and concentration-dependent effects of L-NAME suggest that IC NO is involved in the transmission of acoustic input to the auditory cortex. Stimulation of NOS activity under our experimental conditions might be due to a rise in intracellular Ca²⁺ levels induced by NMDA-receptor activation.

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EFFECT OF BIOTOXINS IDENTIFIED FROM THE SEAWEED *CAULERPA TAXIFOLIA* ON TO THE NERVOUS SYSTEM.

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The tropical seaweed *Caulerpa taxifolia* (Vahl) C. Agard has been accidentally introduced into the Mediterranean Sea starting from 1984. The alga produces a cluster of potentially toxic substances such as mono- and sesquiterpenes together with other secondary metabolites as terpenes. The mechanism of action of these toxic compounds are mostly unknown. In order to study whether the metabolites of *Caulerpa taxifolia* are active in the nervous system, we have tested crude extracts and purified fractions on segmental ganglia of the leech *Hirudo medicinalis* by means of both electrophysiological and neurochemical approaches.

We have tested the effects of crude chloroformic extracts on the electrophysiological activity of sensory neurons of the leech. We have observed: a) a paroxysmic firing in N cells, b) the appearance of frequent and sustained IPSPs on spontaneous activity of T cells, c) the reduction of the amplitude of the afterhyperpolarization (AHP) in T neurons. These changes are similar to those ones induced by serotonin (5HT). In T cells 5HT produces a reduction of the AHP by modulating the Na⁺/K⁺ ATPase: the modulation of this pump represents a new molecular mechanism underlying non associative learning processes in invertebrates.

When segmental ganglia were incubated with 2mg/l crude chloroformic extract, 32P-incorporation was visible into the phosphoproteins with molecular weight of 100, 50 and 20 kDa. These proteins whose level of phosphorylation increases after *Caulerpa* treatment are also more phosphorylated after the incubation with the endogenous neurotransmitter 5HT. The effect of the extract and the monoamine application seems to be additive. The serotonin-like effect observed following *Caulerpa* extracts treatment suggests that these seaweed toxins might play a role in learning processes.

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MOLECULAR AND KINETIC ANALYSIS OF THE INTERACTIONS BETWEEN SYNAPSIN I AND SYNAPTIC VESICLES

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Synapsin I is a synaptic vesicle-associated protein which regulates the efficiency of neurotransmitter release in a manner dependent on its phosphorylation by Ca²⁺/calmodulin-dependent protein kinase II (CaM kinase II). Synapsin I tightly binds to the membrane of synaptic vesicles (SV) and interacts with actin filaments. The binding of synapsin I to SV appears to involve two sites: the hydrophobic NH₂-terminal "head" of the protein interacts with membrane phospholipids and the COOH-terminal "tail" binds to a SV-associated form of CaM kinase II. Using non-perturbing techniques such as fluorescence resonance energy transfer (FRET) between SV labeled with rhodaminated phospholipids and synapsin I labeled with fluorescein on cysteine residues, we have investigated the topology and kinetics of these interactions and their sensitivity to site-specific phosphorylation of synapsin I. Specific FRET was analyzed by exciting fluorescein at 490 nm and measuring either the quenching in fluorescein emission or the increase in rhodamine fluorescence. Synapsin I interacts with both acidic phospholipids and the hydrophobic core of the membrane and the latter interaction accounts for the majority of the binding to the phospholipid site. The binding to synaptic vesicles had a very fast kinetics with time constants of 2.4 sec for the association and of 13.7 sec for the dissociation at 20°C. The calculation of the respective k_{on} and k_{off} allowed a kinetic evaluation of the dissociation constant (K_D = 10.6 nM) which was in close agreement with the value calculated from equilibrium binding studies. Phosphorylation of synapsin I by exogenous or endogenous CaM kinase II dramatically reduced the specific FRET between synapsin I and SV, while phosphorylation by cAMP-dependent protein kinase was ineffective. These data suggest that the reversible interactions between synapsin I and SV occur very rapidly within the nerve terminal and support the hypothesis that the state of phosphorylation of synapsin I regulates the availability of synaptic vesicles for exocytosis.

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