A Relationship Between Oxytocin and ATP in the Hypothalamus

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Magnocellular neurons of the hypothalamus found in the supraoptic nucleus and the paraventricular nucleus are the primary producers of oxytocin (OT). The majority of this secretion travels in axons of the infundibulum to the posterior pituitary where it is released into the pituitary portal system as a hormone.

OT and OT receptors (OTRs) are found in many locations in the central nervous system. Many central synapses release OT as a neurotransmitter. Some of these locations are the hypothalamus , midbrain, pons, medulla , spinal cord and cerebral cortex, including the limbic system and basal ganglia. OT release has been identified in visceral organs such as the stomach, small intestine, kidney, heart and pancreas. (Parks ,Haldar, unpublished) OT has been demonstrated to be release from epidermal cells after stimulation with ATP [1]. The extensive anatomical presentation of OT and OTRs indicates equally extensive physiological roles for OT in many physiological pathways.

The OT molecule is a nonapeptide which contains one disulfide bridge between the Cys in position 1 and the Cys in position 6. Oxytocin is a small peptide with a molecular weight of just over 1kd, approximately 1007. The molecular weight of OT in the posterior pituitary is usually higher due to the fact that OT is secreted with an attached carrier molecule, neurophysin (OT-NP).

The classic hormonal activities of OT eg. parturition, and milk ejection are well known. As a neurotransmitter in central synapses OT mediates higher order behavioral activities. Maternal behavior is closely associated with OT release in cortical synapses. Complex social interactions, such as monogamy have been related to OT and OTR activity [2]. Interactions between OT and digestive hormones , such as gherlin and leptin indicate a linkage of OT with feeding behaviors such as satiety or the initiation of feeding [3]

ATP(Adenosine Triphospate) and the mitochondria, maybe important in the regulatory mechanism that controls the release of OT as a neurotransmitter in the spinal cord of rats (unpublished. Chowdhury 2004). Rotenone (RT) and potassium cyanide (KCN), both classical inhibitors of the electron transport chain, strongly inhibited OT release from spinal cord synaptosomes. These results suggested that the mitochondria and ATP are important in the mechanism that regulates OT release from spinal cord .

There are a large number of receptors that have been demonstrated to mediate the actions of ATP, these are the Purinergic receptors. There are two families of purinergic receptors , the P2X family and the P2Y family. Both receptors regulate calcium transport . The P2X group is made up of ionotrophic (directly gated) Ca^{++} receptor /channels. They are designated P2X 1, 2,3 ,4 , 6, a heterodimer of the 4 and 6 isoforms has also been reported. The preferred ligand is ATP. The classical antagonist for type P2 X1, X2, and X3 is

PPADS(Pyridoxal-phosphate-6-azophenyl-2',4'-disulfonate) and the agonist for the X4 and X6 is ATPγS , a slowly hydrolysable form of ATP

The P2Y family members are metabolic (indirectly gated) receptors. This is a member of the seven transmembrane receptor group or a G protein coupled receptor (GPCR). The second messenger system employed is usually diphosphoglycerate (DPG) which associates it with the PKC pathway. The preferred agonist of P2Y receptors is ADP . The CCK – B receptor found central synapses are also a member of this large group of GPCRs. [4}

Studies by Song and Sladek done with hypothalamic/pituitary preparations suggest a important regulatory role of either or both purinergic receptor systems being responsible for the direct stimulation of anti-diuretic hormone (ADH) release in these preparations that was also inhibited by the P2X antagonist PPADS [5]. Hegg demonstrated that transduction of odorant stimuli in olfactory epithelium was dependent on P2X activation. The transduction could be blocked by a 25uM dose of PPADS . This inhibition also resulted in a decrease in Ca++ conductance. These observations suggest a relatively important role for ATP as a neurotransmitter or co-transmitter in central synapses. [6]

The role of ATP as neurotransmitter or secondary modulator may be relatively common in nervous system. [7] showed that ATP induced epinephrine release while being is regulated by PKC. [8] showed ATP to be a neuro-endocrine regulator in pituitary gland [9]. Buller reported that vasopressin (VP) release in brainstem was ATP receptor dependent.[10] Thoadec and Thirion suggested that ATP and adenosine are modulators in the release of neuropeptides from the pituitary . [11]

The important regulatory function of Ca^{++} in neurosecretion and endocrine secretion is well established. Classic experiments from neuroscience done by Katz, Miledi, Hauser ,Llinas and others have demonstrated that neurosecretion is Ca^{++} dependent. The amount of secretion or quantal release , is directly proportional to the cytoplasmic concentration of Ca^{++} at the time the cell is depolarized. These researchers also demonstrated that the required calcium can come from extracellular or intracellular storage. [12] In neural cells a voltage sensitive Ca^{++} current was measured that was pharmacologically distinct from the Na+ current [13]. Turner and Goldin showed $^{45}Ca^{++}$ labeled uptake by Ca^{++} channels of synaptosomes that could be blocked by nifedipine and verapamil [14]. In 1992 Bertil Hille wrote about the ultimate importance of Ca++ in neurotransmission this way in his book , Ionic Channels of Excitable Membranes. "…… excitable cells translate their electricity into action by Ca++ fluxes modulated by voltage sensitive Ca++ channels ……. Ca++ channels are the only link to transduce depolarization into all the nonelectrical activities controlled by excitation. Without Ca++ channels our nervous system would have no outputs" [15].

The exocytosis of OT follows these classical rules . The work of Cazalis demonstrated that OT secretion was Ca++ dependent. He also showed that ATP potentiates the release of OT to a greater extent than GTP . He found that the phorbol ester , TPA , a strong activator of the PKC , increased the amount of OT released. This study showed that OT release was not affected by external Ca++ concentrations [16].

The mitochondria play a fundamental role in many essential cellular processes. The mitochondrion is involved in

many important physiological activities such as in Kreb's cycle and electron transport chain reactions for the synthesis of ATP,as well as in metabolic control and apoptosis [17, 18, 19]. The mitochondrial activity that has been shown to be very important in neurosecretion is Ca++ homeostasis. It was proposed by Alnaes and Rahaminiff the role of mitochondria in transmitter release from motor nerve terminals, that mitochondria were one of the organelles responsible for Ca++ homeostasis in nerve terminals, and therefore, directly responsible for triggering neurotransmitter release.[20]

David et al.[21] provided direct observation of the role of mitochondria in intracellular Ca++ storage and regulation. After treating the cytosolic Ca++ with Oregon Green BAPTA and the mitochondrial Ca++ with rhod-2 the presynaptic terminals of the neuromuscular junction were scanned with a laser confocal microscope. They saw that if mitochondrial Ca++ uptake was inhibited, the cytosolic Ca++ concentration would obviously rise, which probably corresponds to the observed transmitter release.

The essentially required step for all neurotransmission is the docking of vesicles to the plasma membrane for delivery of the neurotransmitter into the synaptic cleft or extracellular space. In this fundamental process , Ca++ plays a vital role.

Peptide neurotransmitters , like OT , are synthesizes in the standard way by trancription and translation. They are transported down the axon to the release site via axonic transport. Peptide neurotransmitters are stored in dense core vesicles at the release sites tethered to the cytoskeleton, usually by actin. These tethering proteins are called synapsins which are substrates for Ca++/calmodulin –dependent protein kinase. Depolarization of a nerve terminal or storage site causes the cytoplasmic concentration of Ca++ to rise in this region. This activates the Ca++/calmodulin –dependent protein kinase, which phosphorylates the synapsins. Phosphorylated synapsin causes the vesicle to be released from it's tether on actin and the vesicle is now free to dock with the membrane. Hydrolysis of GTP by Rab drives the targeting of the vesicle to it's docking location on the membrane. The proteins required for docking are a v-snare , usually synaptobrevin and a t-snare , usually SNAP-25 and or syntaxin. After docking is completed then fusion of the membranes proceeds with the exocytosis of the neurotransmitter. ATP hydrolysis drives fusion and exocytosis of peptide [21].The amount of ATP needed to energize this set of events is very high, the actually concentration is in the millimolar range [22].
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II. OT release from hypothalamic preparations.

The factors that effect OT release from many synapse has been studies in Dr. Haldar's lab at St. John's University for many years . The brainstem, spinal cord , posterior pituitary and the hypothalamus were the primary anatomical sites used to study the mechanisms of OT release. The work I did in Dr. Haldar's lab specifically examined the physiology of OT exocytosis from hypothalamic preparations. Two different systems used , slice explants and synaptosomes.

In the initial experiments, slice explants of neonatal rat hypothalami were used . These results showed that CCK stimulated OT release. The slices were incubated with or without CCK. RIA's were done on the collected media . The cells that were incubated with the CCK had a large concentration of OT as compared to unstimulated control slices. (Altimari, Haldar . unpublished data). This finding was very interesting due to the fact that the

media used in the experiments was calcium free to the greatest extent possible. The next experiments, also in calcium free media were with the phorbol ester , PMA, It is know that PMA is a mimic of DAG and therefore activates the PKC pathway on organelle membranes. 1.6 nM PMA caused 3 times more OT release than control (Altimari, Haldar . unpublished data).

The 50nM CCK and ATP release studies reproduced in hypothalamic synaptosomes demonstrated positive results . The results of these experiments showed that ATP stimulated OT release and that mitochondria was the sequestering compartment for the required calcium . See ATP modulates Hypothalamic Oxytocin Exocytosis via Mitochondrial Calcium Release for details [23]. Here it was demonstrated that OT release was significantly inhibited by CGP37157, a specific inhibitor of the mitochondrial Na+/Ca++ antiporter. This drug selectively inhibits the efflux of Ca++ ion from the mitochondrion with resultant inhibition of neurotransmitter release.

Purinergic receptors (P2) have been demonstrated in many neural system. Initially, experiments were designed to test whether a P2 receptors was involved in ATP mediated OT release. In almost all trials done in synaptosomal preparations, OT release was robustly stimulated by ATP went compared to other purinergic ligands tested. Some of these ligands were ADP, GTP and ATP γ S. ATP stimulation experiments done with prior or simultaneous treatment with PPADS at different concentrations of PPADS up to 1mM and incubation times up to 3 minutes showed no inhibition of 50 nM ATP mediated OT release. These data suggest that ATP stimulation of OT release in hypothalamic preparations did not seem to be mediated by a P2X receptor. In a large number of trials the known, classical receptor antagonist of P2X receptors did not inhibit OT release in hypothalamic synaptosomal preparations. This is a novel observation and is contrary to published reports in other systems. These data are presented in ATP modulates Hypothalamic Oxytocin Exocytosis via a novel non-Purinergic Receptor Mechanism [24].

In summary, the results that were observed suggest that ATP is working as a regulator in the transduction of OT exocytosis in this system. Other purine nucleotides did not demonstrate the same consistent and robust stimulation of OT release of 50 nM ATP. Tests with GTP also showed weak stimulation. This data plus the small concentrations of ATP used , 50nM gave the maximal response in dose experiments, suggests that the ATP added to the system is not an energy source driving exocytosis machinery, in other words it is not a simple metabolic effect , but rather acting as a step in the transduction system. The model that can be proposed is electrophysiologic. The negative results reported suggest against a purinergic receptor mediated mechanism. The positive experimental results support a electrophysiologic mechanism that involves depolarization of calcium channels with Ca++ release from mitochondrial sequestration.

Three sets of experiments that were performed during the course of my studies with hypothalmic preparations support this suggestion.

1. KCl strongly and consistently stimulates OT from spinal cord and hypothalamic synaptosomes and in slice explant cultures. The mechanism of KCl is electrophysiologic. It directly depolarizies Calcium gated channels in cells. (Altimari, Haldar, Chowdhury unpublished data)

2. PMA, an analogue of DAG, stimulates OT release from slice explants cultures to the same extent as CCK, acting

as the positive control . PMA, acting like DAG stimulates the PKC pathway which results in opening of voltage sensitive calcium gates in the membranes of many organelles including mitochondria. (Altimari, Haldar , unpublished)

3. CGP37157, a selective inhibitor of the mitochondrial calcium/sodium antiporter results in strong inhibition of OT release. [23]

Dedication

This Brief review is dedicated to my mentor and teacher, Dr. Jaya Haldar.
Dr. Jaya Haldar was truly my mentor, she believed in me even when I didn't.
She guided me in every step and never lets me come even remotely close to failure.
She protected and directed me with the care and diligence of a mother who watches over her children , protecting them from harm but always sending them towards the point when they can work and think not like her but as free thinking, reflective and truly productive individuals.
I owe my life and career to the work she did to make me what I am. This essay is a very small thank you to her for all she has done for me and all the students she taught and mentored for decades.

My reflection of her is not as my Ph.D mentor but more like the superhero of a novel. Her story is one of great achievement that started from a little girl born in Calcutta, India in the 1940's that grew up to attend The University of London were she started, completed and defended her dissertation on the neuroendocrinology of oxytocin in 2 years. She came to the United States as a young wife and mother , where she worked at Columbia University, then on to St John's University where she taught hundreds of undergraduates and dozens of graduate students, many who went on to high levels in academia. I had the profound honor to be her last Ph.D student. I live everyday trying to work and learn enough to achieve, in an attempt to properly repay her for all she did for me. I doubt that I will be able in as much as the debt is too large to be repaid in such as short time as one man's life.

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