BIO F211_GROUP13_1990 & 2000

BIO F211 – Take Home Assignment

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The Nobel Prize in Physiology or Medicine 1990: Joseph E. Murray and E. Donnall Thomas "for their discoveries concerning organ and cell transplantation in the treatment of human disease"

The Nobel Prize for Physiology And Medicine in the year 1990 was jointly awarded to Joseph E. Murray and E. Donnall Thomas for their discoveries concerning "organ and cell transplantation in the treatment of human diseases." Joseph E. Murphy is credited to perform the first successful human kidney transplant on identical twins, named Richard and Ronald Herrick in the year of 1954. He also worked on rejection following organ transplantation and immunosuppressive agents. E. Donnall Thomas researched on diminishing the effects of Graft-Versus-Host (GVH) reaction and bone marrow transplantation. He showed that using radiation and chemotherapy, a body's immune system rejection can be lowered facilitating transplant of donor's bone marrow cells through blood transfusion.

In 1953, Billingham, Brent, and Medawar showed that immune tolerance can be deliberately produced. They showed that tolerance in skin allografts can be induced by injecting fetal or prenatal mice with immunocompetent spleen cells from adult donors. Billingham also discovered that profusion of immuno-competent hematopoietic cells lead to graft-versus-host reaction if there is no histocompatibility (the close genetic relationship) between donor and patient. However, despite Medawar extensive work on timing, morphology and immunological features of the rejection, no antibody was detected. This led him to the conclusion that a biological force will always and forever inhibit organ transplantation. His contemporaries largely worked on defining these biological forces leading to the discovery of HLA (Human Leukocyte Antigens) on the cell surface. These antigens on the cell surface of the transplanted organ are recognized by the immune system of the recipient leading to rejection of the transplant organ and the subsequent reaction was termed as "Graft-Versus-Host reaction. It became evident that to master the transplantation, immune response in cells needs to be suppressed. Joseph E. Murphy discovered that by using ionizing irradiation and later, by use of cell proliferation inhibiting cytotoxic drugs azathioprine, risk of rejection can be subdued. On the other hand, E Donnell Thomas used the drug methotrexate to pave the way for bone marrow cell transfer between donor and recipient.

Joseph E. Murray—Nobel Prize for Organ Transplantation

Discovery And Literature Review

Prior to Murray's renal transplantation in identical twins, Medawar and Rupert Billingham showed that most cows accepted skin grafts of their identical twins. It was reasoned that it was due to the persistence of chimerism not only in RBCs but also in stem cells perpetuating the RBCs. Barely 14 months later, Murray performed his famous transplantation which validated that human bodies also accept organs (in this case, kidney) if recipient and donor are identical twins. It was one of its kind as the recipient Richard lived normally for 8 more years following the operation. For prior kidney transplants in humans, recipients barely survived one year. In retrospect, this was of little scientific significance as acceptance of skin grafts between identical twins was established before. But, the profound media coverage acted as a stimulus for surgeons to put more effort into the clinical transplantation. One of the main limitations was that since the induction of chimerism by the help of neonatal treatment is not possible in humans, this approach was of limited use. Next year, Richmond Prehn and John Main showed that if the immune system in mice can be weakened, chimerism can be introduced by inoculating bone marrow cells. This was followed by vast research by Murray and his team on immunosuppression by total body irradiation (TBI). The challenges they had were:

1. Genetics of no other species as the mouse was studied.
2. Existence of distinct species variations independent of the genetic variations
3. Variation in test systems. In mice, it was generally skin or tumour graft, whereas vascularized whole organs were used in the case of humans.

For conducting their experiment, they exposed some patients with 600, 700 r TBI followed by bone marrow infusion operating at 2.0 million volts source. For other sets, patients were exposed to sub-lethal, non-marrow requiring TBI at 250 kV potential source. Prospective donors were selected from those sharing the greatest number of blood antigens. Among the 12 irradiated recipients, only one survived. The survivor was not given bone marrow cells and

received the kidney from his fraternal twin, showed adequate renal function before passing 20 years later. This was a breakthrough in terms that the genetic barrier to transplantation has breached for the first time. It proved that if immune suppression is successful, organs can be transplanted in cases other than identical twins. This also proved that bone marrow infusion is not essential for prolonged survival of kidney allografts, TBI alone can sustain it for prolonged periods and hence, eliminating the need for chimerism. Later, Kuss and Hamburger showed that adrenal cortical steroids can be used as a supplement to TBI. In the following three years, it was proved by various research that variable degrees of grafts acceptance achieved by TBI can also be replicated through the use of drugs alone.

In 1959, Robert Schwartz and William Dameshek, showed that 6-mercaptopurine (6-MP), an anti-cancer drug prepared by Gertrude Elion and George Hitchings at the Wellcome Research Laboratories in New York can prevent rabbits to produce antibodies in response to foreign proteins. This drug was also effective in prolonging foreign skin graft in the rabbits. Murray, along with Calne then researched on a derivative of 6-MP, called azathioprine. Although 95% of the canines injected with this drug died within 100 days due to rejection or infection, it was observed that in some cases, permanent allograft acceptance was recorded following 4-12 months of immunosuppression. This number was still better than TBI performance with or without bone marrow. Some of these animals survived for 6 months, paving way for azathioprine's clinical trials focusing on drug combination with azathioprine. Following the failure of adrenal cortical steroids, Murray opted for cytotoxic agents azaserine and actinomycin C. After many more trials, reversal of rejection in human homografts was achieved by supplementing azathioprine with high doses of prednisone (200 mg/day) to commence immunosuppression. Also, self-induced tolerance of kidneys with these cytotoxic drugs was also proven later. The subsequent and diminishing need of these drugs made it clear that recipients could start an unrestricted and normal life after a specific period of transplantation.

Current Status And Future Trends

Murray's contribution in renal transplantation and immunosuppression inspired attempts for other organ transplants. Presently, tens of thousands of kidney transplants are done every year with increasing chances of graft survival and is today about 80%. Liver, heart and lung transplantation soon followed and it was proved that every organ can show immunosuppression. Murray's work also led to extensive discoveries on better and efficient immunosuppressive drugs such as protein therapies calcineurin Phosphatase Inhibitors (Cyclosporine, Tacrolimus), and Antiproliferative Agents (Azathioprine and Mycophenolate Mofetil). It also led to research on immune suppressant mechanisms in cancer patients and need to devise better ways that chemotherapy and antiproliferative drugs. In the last ten years, many studies have suggested that stable and functional kidney grafts may contain n tubulointerstitial inflammatory infiltrates, histological similar to clinical acute cellular infiltrates noticed in the biopsies of rejecting grafts. Research is underway to test the hypothesis that these inflammatory infiltrates are the reason why graft survival is not prolonging despite the use of heavy immunosuppressants. This phenomenon was described as subclinical acute rejection (SCAR). The validity of this hypothesis can lead to development of more refined, precise, sensitive and specific non-invasive methods by using innovations in genomics, proteomics, metabolomics and gene chip techniques and ultimately optimization of present immunosuppressive therapies. Another challenge in organ transplantation is poor median survival rate in case of lung transplant. It is attributed to infection, graft failure, chronic lung allograft dysfunction in later stages. Adding to these complications, the lung is the only transplanted organ which is directly exposed to environmental agents. Therefore, it has different immune mechanisms rendering present immunosuppression drugs less effective. Another major challenge in post-transplant therapy is to achieve transplant immune tolerance i.e. a state in which recipient's immune system no longer considers allograft as foreign. It can happen by achieving mixed chimerism: state where donor and recipient hematopoietic cells can co-exist leading to donor-specific tolerance in the recipient. Various myeloablative and non-myeloablative procedures are going through rigorous clinical trials to test the same. Also, studies in last century were highly constrained to cell mediated immunity but risks associated with antibody mediated rejection (AMR) are becoming clear. Definitive diagnosis of AMR and its efficacy in reducing lung transplant rejection is a matter of debate. AMR therapies involve plasmapheresis, intravenous immunoglobulin (IVIG), rituximab, bortezomib and the newer agent carfilzomib. Bortezomib and carfilzomib are two proteasome inhibitors which allow ubiqutinated proteins in cells to accumulate leading to apoptosis and decrease in antibody levels. An overview of this medicinal field indicates that the future

will be focused on three main aspects: One, increment in frequency, safety and result of traditional organ transplant procedures. This includes expanding the donor pool, preservation of organs etc. In solid organ transplant- research is ongoing on prolonged allograft survival while minimizing the harmful effects of immunosuppressive drugs. Second will be the development in emerging transplantations such as uterus, face and composite tissue. The gap and supply in the organs for transplantations has led researchers to look for other alternative solutions and therapeutic modalities.So, third will be the research for bioartificial devices which reduces immunosuppressant dependency and subsequently replace organ transplantation in future. Xenotransplantation, i.e transplantation of organ, tissue or cell between different species. Researchers have identified that Galactosyl-α-1-3, galactose (GAL) is the most important antigen and pigs as most suitable animals for xenotransplantation. This field is also witnessing huge opportunities in organ bioengineering, regenerative medicine, 3D bioprinting and machine perfusion.

E. Donnall Thomas – Nobel Prize for Discoveries concerning organ and cell transplantation in the treatment of human disease.

Literature Review And Discovery

E. Donnall Thomas research in GVH and bone marrow transplant was the result of his quest for treatment of leukemia. Chronic myelogenous leukemia (CML) was particularly of his interest as it was not curable using chemotherapy and its unique cytogenetic marker.He followed a simple idea, if leukemia is a disease in bone marrow, and if radiation removes bone marrow cells, can irradiation destroy leukemic host and regenerate the host;s bone marrow by aid from transfusion of healthy blood cells from other donors. In 1959, Don showed that marrow from a healthy identical twin can regenerate the recipient's marrow, thus elucidating genetic compatibility between the donor and the recipient. This was replicated by using a dog leukocyte agent in the laboratory. In 1969, this was followed by a transplantation of HLA-matched bone marrow in a patient suffering from late, acute leukemic phase of chronic myelogenous leukemia. However, the patient showed symptoms of GVHD soon after transplant and when treated with immunosuppressants, caught a viral infection and died soon after. This failure posed a question of balancing immunosuppression and GVHD. This also made them realise that this therapy can be extended to acquired as well as genetic bone marrow conditions forming the basis of future regenerative medicine. Even with HLA matching sibling donors, GVH was seen in almost half of the transplants. Don's research on canines for GVH showed cytotoxic drugs, MTX (methotrexate), and glucocorticoids partially effective in the treatment. It was proved that MTX inhibits dihydrofolate reductase and production of thymidylate and purines, suppressing T-cell response, proliferation and expression of adhesion molecules. Powles in 1978 described the effectiveness of cyclosporin A against GVHD in man. Don and his team added cyclosporine for 6 months following a short course of MTX after the patient received an allogeneic HCT(hematopoietic cell transplant). In the late 1970s, Donnell was able to demonstrate greatly improved overall survival rate in the transplants for leukemia in first remission or relapse. Among 19 patients with acute myeloid leukemia in first remission, 8 people survived and lived up to 18-21 years following the transplant. Thomas demonstrated the efficiency of the HCT in case of non-malignant disorders other than immunodeficiency diseases such as aplastic anemia. Using a combination of CY, antithymocyte globulin, cyclosporine and MTX dramatically improved the results. To prepare immunosuppressants and antileukemic reagents, total body irradiation was an obvious choice due to its wide use in transplantation and sensitivity of leukemic cells. Thomas and Ferrebee, in an effort to establish graft quickly, single-time exposed patients with advanced leukemia. 1000 rad TBI was administered at 7 rad/min. However, the few patients who were successfully engrafted showed early recurrence of leukemia. This led to the conclusion that TBI alone will be insufficient to destroy all leukemic cells in the bone marrow. His team then injected CY, a known anti-leukemic drug two days before TBI. This regimen succeeded in producing healthy allogeneic cells in the recipients. As the preparatory regimen and supportive care improved, immediate transplantation in people with advanced leukemia no longer remained the need,and the superiority of fractionation of TBI was established.

Current Status And Future Trends

E. Donnall Thomas discovery of bone marrow transplantation is now the standard treatment for leukemia. In the 1970s, bone marrow transplantation was considered a failed therapy and the only option was chemotherapy and

radiation, which killed the cancerous cells but left no scope for the regeneration of the bone marrow blood cells. However, Don's work recognized the importance of matching donors with recipients, on immunosuppression before transplant, and subdue reactions of GVHD by using drug methotrexate, proved viability of bone marrow transplantation and its transformation into routine clinical procedure for certain malignant as well as non-malignant conditions such as thalassemia, aplastic anemia etc. Bone marrow transplantation and its sister therapies, such as blood stem cell transplantation has alleviated survival rates from 0 to 90 percent in various forms of blood cancer. Before his work, acute lymphocytic leukemia had a very high mortality rate. Due to his research, the survival rate of lymphocytic leukemia patients with good HLA has reached 80%. E. Donnall Thomas subsequently came to be regarded as "Father of Bone Marrow Transplantation" due to his extensive work on hematopoiesis, tissue typing, and immunology.

The success of HCT can be grouped into three advances in - 1) Reduction of transplant-related morbidity and mortality 2) Expansion of donor options 3) reduction in post-transplant relapse. There have been significant advances in mortality after allo-HCT due to decrease in organ damage, better prophylaxis and treatment strategies for infectious diseases post-transplant. Widely developed radiologic studies, micro-biologic and histopathologic techniques, and fungal biomarker assays has made diagnosis and treatment of invasive fungal and viral infections much simpler. GVHD, however, is still one of the major complications. Significant heterogeneity, complex pathogenesis, a lack of well-defined therapeutic targets have resulted in no breakthrough in the past two decades. However, frequency and severity is gradually decreasing in GVHD owing to selection of better matched unrelated donors for high resolution HLA typing, development of non-myeloablative conditioning, and decrease in TBI use. It is under investigation that use of post-transplant cyclophosphamide as GVHD prophylaxis and selection of unrelated donors in allo-HCT yields better outcome. Limited availability of suitable HLA identical donors is also a concern. For patients lacking a suitable donor, umbilical cord blood transplantation was the only option but a small number of progenitors in cord units and slow immune reconstitution puts the recipient at greater risk. Several novel approaches are working to overcome this HLA disparity, notable among them is haploi-dentical (half-matched) HCT followed by post-transplant cyclophosphamide. Investigation and clinical trials are also going on decreasing relapse risk by inducing deeper responses to anti-cancer therapy, enhancing cytotoxic potential of conditioning regimen and maintenance therapy using lenalidomide or bortezomib. Until recently, there were no proper laboratory tools to diagnose development and severity of acute GVHD. This landscape is changing with identification of several serological markers like TNFR1, ST2, and Reg3α. Further development and validation of biomarker based algorithms is expected to provide a clear picture of acute GVHD cases. Identification of novel key targets and signalling pathways like Bruton's tyrosine kinase (BTK), Janus kinases (JAKs) in chronic GVHD pathogenesis has taken place. Research is also going on the relationship between GVHD and commensal bacteria, especially gut microbiota. Research on development of effective post-transplant maintenance approaches is also underway. Immune escape, one of the key factors in relapse after allo-HCT is a focal point of enhancing the graft-versus-tumor effect. Advent of molecular technologies such as genomic breakpoint cloning, whole genome sequencing, single nucleotide polymorphism profiling, RNA sequencing , and whole exome sequencing have led to better recipient management and are contributing in target selection for GVT responses.

The Nobel Prize in Physiology or Medicine 2000: Arvid Carlsson, Paul Greengard and Eric R. Kandel "for their discoveries concerning signal transduction in the nervous system"

Eric Kandel- Nobel Prize for discovering the central role synapses play in memory and learning

Eric Kandel, is an Austrian-American neuroscientist, and a biochemistry professor at the Center for Neurobiology and Behaviour, at Columbia University, New York. He won the Nobel prize in Medicine in 2000, for his studies on the nervous system of a sea slug. By conducting this experiment, he explained how the nervous system and the synaptic function are vital for memory and learning of organisms. He also successfully explained the concepts and causes of Short term memory (STM) and Long term memory (LTM) in animals and humans both.

Literature Review And Discovery

Learning is the process by which organisms acquire information about various things, while memory is the form in which the information gets stored.

Historically speaking, all kinds of research regarding learning, began towards the end of the nineteenth century when two scientists Edward Thorndike and Ivan Pavlov came up with the concepts of: instrumental conditioning (or learning by reinforcement) and classical conditioning. Thorndike observed that a cat would quickly learn how to escape a cage, if it were to be awarded with some food, every time it escaped successfully. Therefore, this was learning by reinforcement or instrumental conditioning, as the cat learned a new behaviour quickly so as to be rewarded every time. Another scientist Pavlov, carried out experiments on dogs, to examine classical conditioning. Every time before offering food to the dog, he would always first ring a bell. Thus, the dog started identifying the ringing of the bell with the imminent arrival of food, and started salivating. So, in classical conditioning, the unconditioned stimulus of salivation occurs due to the conditioned stimulus of bell ringing.

When the behaviours of organisms change, it's mostly due to certain changes in the strength of the connections (synapses) between adjacent neurons. In the 1940s, two scientists Jerry Konorski and Donald Hebb explained that, if coincident activity occurs in two adjacent neurons, then their synapse gets strengthened.

Hebb also discovered that there are two distinct forms of memory: short term memory and long term memory. However, no scientist had yet been able to explain how these two kinds of memories were created in organisms, and what were their precise differences. Also, no one was yet able to explain the phenomenon of 'learning', i.e., how the nervous systems of organisms acquire and process information regarding various things. And these were precisely the questions that Kandel's studies explained. This was what won him the Nobel prize and this is why his studies revolutionized the field of neuroscience.

Eric Kandel took Hebb's studies forward when he explained STM and LTM via his sea slug experiment. To study the formation of memories in organisms, Kandel started by examining the nervous systems of certain mammals. But he soon dropped this idea, as he found that their nervous systems were quite complex to understand. He therefore decided to study the nervous system of a sea slug (Aprysia californica), as it had only about 20,000 neurons, of which many were large sized and identifiable. This made it easier to study and record the activities of these neurons.

A sea slug is capable of showing a typical gill-withdrawal reflex behaviour. This means that, a sea slug would instinctively withdraw its gills if it is touched anywhere on the body.

By studying this reflex behaviour, Kandel could study the sea slug's nervous system and its entire neuronal circuitry. Kandel observed the gill withdrawal of the sea slug and the corresponding activities of its neurons. Hence, he could explain the exact behaviour and interactions of neurons and how they caused this reflex behaviour.

Kandel also said that some stimuli can amplify the reflex of the sea slug. The strengthened reflex is the organism's 'learning', as it can maintain itself for a few days. The sensory nerve cells that sense the stimulus, are connected to the nerve cells that activate muscles, which in turn leads to the reflex. Learning occurs whenever, the synapse connecting these neurons gets amplified.

However, most of the learning that organisms do, doesn't last forever, since memories fade away gradually.

Depending on the amount of time for which learning is retained, memory is of two types: short term memory (STM), which lasts for a few minutes or hours, and long term memory (LTM) which last for days or months. STM is caused due to weaker stimuli, while LTM is caused by stronger stimuli.

A STM can be produced with the help of few trials or just a few interactions with the stimuli, however, to create a LTM, the stimuli would need to interact with the organism for a sufficiently long time.

Mechanism for STM: The amount of calcium ions entering into the nerve terminal increases since some ion channels get affected and some ion channel proteins get phosphorylated, after coming into contact with the stimuli. Due to this, more amount of transmitter gets released at the synapse. The protective reflex also gets amplified in magnitude, and thus, a STM is produced. Hence, the phosphorylation of proteins in synapses can create a STM.

Mechanism for LTM: If the organism comes into contact with a strong and long lasting stimulus, then it develops a LTM. Once this strong stimulus acts on the organism, cAMP molecules (which are messenger molecules) increase in quantity, and so do protein kinase A molecules. Once these messenger signals reach the nucleus, some proteins of the synapse increase in number, while some decrease in number. These changes in protein synthesis can lead to a change of shape and function of synapse, and an increase in size of the synapse. Hence, the synaptic function can long laster, and a LTM is created. In conclusion, it can be said that, LTM requires the formation of new proteins, while STM doesn't.

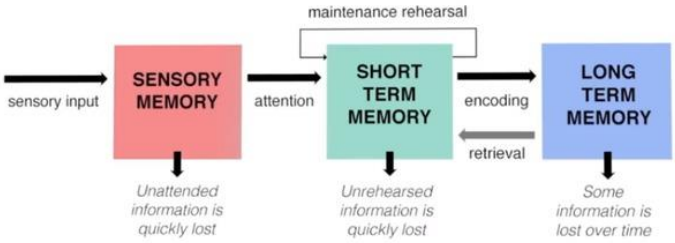
After successfully proving that STM and LTM are both present in the synapse of a sea slug, Kandel went on to prove that similar concepts hold for mammals as well. The memory of mammals is also present in their synapses, and whenever mammals form new memories, synaptic function changes are essential for it.

One more interesting conclusion that can be drawn from Kandel's research is as follows. Kandle found out that, memory retention in organisms happens for a much longer time period if the learning process is carried out in steps/slots, as opposed to learning a lot of things in one go. In the language of biology, if a stimulus interacts with an organism several times within a short time span, then it is ineffective in creating a LTM. However, if the same stimulus repeatedly interacts with the organism at fixed time intervals, then it can successfully create a LTM. This is why cramming information isn't helpful, and education based on rote learning doesn't help much.

Current Status And Future Trends

Kandel's work revolutionised the field of signal transduction in the nervous system by explaining how organisms attain different kinds of 'learnings' and how short term and long term memories are created and stored. He laid down the cellular and molecular basis in our understanding of memory functions in organisms.
Still, some questions in this field remain unanswered. Several complex memory functions are yet to be understood by the scientists. Scientists are yet to figure out how images get stored in our nervous system. We are also unaware of how the brain manages to retrieve and recreate the memory of the past. Building upon Kendal's discoveries, scientists may also attempt to create new medicines which would help dementia patients in improving their memory functions.

The Nobel Prize in the field of Physiology or Medicine for the year 2000, was given to Arvid Carlsson, Paul Greengard and Eric Kandel for their work on the topic of 'Signal Transduction in the Nervous System'. Here in this report we will address. This Nobel prize is peculiar in the sense that under the umbrella of Signal Transduction in the Nervous System, all the discoveries are minutely connected to each other. Therefore, we will unfold the discoveries distinctly.



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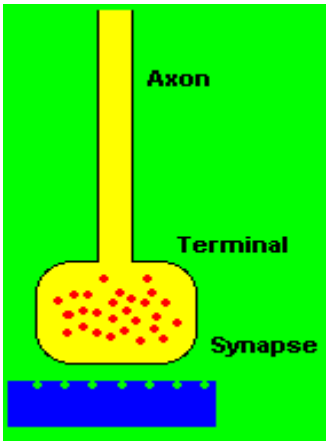
Arvid Carlsson- Nobel prize for his research establishing dopamine as an important neurotransmitter in the brain.

Arvid Carlsson was one of the awardees of the Nobel Prize and got 1/3 prize. He was from the Department of Pharmacology, Göteborg University. He was awarded due to his discovery that dopamine is a neurotransmitter which has great importance in controlling the movements. This discovery established the foundation for the consequent research, which ultimately proved dopamine deficiency as a cause for Parkinson's disease. His research helped in subsequent investigations which were related to the brain.

Literature Review And Discovery

So, we will start with the history which revolves around the neurotransmitters. This phenomenon of neurotransmission had various historical interpretations out of which three are prominent. First, was humoral, which was formulated by the ancient Greeks (Alexandrian School). This theory lasted until the sixteenth century when Galen gave the theory of spiritus animals based on Cartesian conceptions. This theory was prominent in the seventeenth and eighteenth centuries. Then in the nineteenth century, the electrochemical interpretation came which can be divided further into an electrical hypothesis which was dominant in the nineteenth century and the current chemical hypothesis, since the sympathetic simulation concept of Elliott and Langely came in 1904. Ramón y Cajal by Histological examinations in the late 19th century found that a 20 to 40 nm gap is present between neurons. This was what now known as the synaptic cleft, was discovered. Before the discovery of Ramon y Cajal, scientists assumed that majority of synaptic communication in the brain was electrical. From then the point of interest changed from electrical transmitter to chemical transmitter as that gap was to be traversed by a chemical transmitter. After 1904 due to the work of Langley and Elliott people realised that the signal transduction process works on the principle of chemical stimulant and receptors. Since then, the process of discovery of the different neurotransmitters and neuro-receptors and analyse the new interpretations postulated concerning the neurotransmission concept at the dawn of the twenty-first century. This is also called the concept of neurochemical transmission.

The first neurotransmitter discovered was the Acetylcholine, by Austrian scientist Otto Loewi in the year 1921. After that many more neurotransmitters were discovered. A major breakthrough was the discovery of Dopamine as the neurotransmitter.



[Ref: Arvid Carlsson: 5]

Dopamine – an important transmitter: Dopamine was earlier considered to be the precursor of a transmitter called noradrenaline, and it's derivatives were an intermediate in the formation of norepinephrine. In the late '50s, co-ordinate efforts were lead by Kathleen Montagu at Hans Weil-Malherbe lab and Arvin Carlsson at Lund University (Sweden). These pioneering efforts lead to the initial discoveries that would collectively suggest dopamine's function as a neurotransmitter in the human brain. Arvid Carlsson developed an assay that helps in measuring the concentration of dopamine in the brain with high sensitivity. He observed that dopamine was concentrated in that region of the brain where noradrenaline was not present, these led him to the conclusion that dopamine is a transmitter in itself. Besides, a series of experiments were carried out by Arvid Carlsson on rabbits, where naturally occurring substance, reserpine was used, which depletes the storage of several synaptic transmitters. He concluded that he could reverse the akinetic effects that reserpine caused in his rabbits by treatment of dopamine and norepinephrine precursor, 3,4-dihydroxyphenylalanine (L-DOPA) intravenously and found that it affects the recovery of dopamine but not noradrenaline. The analysis if this experiment proposes that the lack of dopamine may have been responsible for the akinetic state seen in his animals. Eventually, Carlsson's group mapped out where the highest concentration of dopamine was present in the brain. They found that dopamine was found in high concentrations in the striatum, which is the largest component of the basal ganglia. Moreover, basal ganglia played a crucial role in voluntary motor functions. These discoveries helped to shape the hypotheses that dopamine may be a key neurotransmitter in the control of motor function. Arvid Carlsson also showed that the treatment with L-dopa normalized the levels of dopamine in the brain.

Drugs against Parkinson's disease Arvid Carlsson noticed that the symptoms caused by reserpine were similar to the syndrome of Parkinson's disease. Further, it was found that people with Parkinson have low levels of dopamine in the basal ganglia. Nerve cells which produce dopamine in the basal ganglia of the brain, degenerates which cause disorders like tremors while resting, rigidity, stiffness in body parts and akinesia. Here the discovery of Arvid Carlson proved crucial, and the L-dopa was developed as a drug against Parkinson's disease. L-dopa acts as the chemical precursor of dopamine and is converted to dopamine in the brain after the removal of amine from it. Hence, the previously discussed degenerated cells, are lost forever, but L-dopa increases the production of dopamine in the cells, which compensate for the lack of dopamine and normalizes motor behaviour.

Current Status And Future Trends

Dopamine neurones are estimated to be less than 1% of the total neuronal population of the brain but have multiple effects on the functioning of the nervous system. As mentioned above, dopamine is the neurotransmitter involved in the control of movement and Parkinson's disease. Besides, it is an essential antipsychotic or anti-

depressive drug, which was proved by the research of Arvid Carlsson. These were the main reasons behind the Nobel prize award being conferred to him. But after that, various other fields found the dopamine application like the neurobiology and schizophrenia and attention deficit hyperactivity disorder (ADHD). Likewise, it is a crucial element of the brain reward system and in the action of many drugs of abuse. In contemporary times we know about the metabolism of dopamine, dopamine receptor systems and the of dopamine pathways in the brain. But many things are not known about dopamine, which if answered would be beneficial in the cures of psychiatric disorders.
Questions which unfolded as the research on the dopamine proceeded, many were answered, but some question still intrigues the researchers. One such question is that- Is it possible for a neuron to fire properly in the absence of its neurotransmitter? This question arose because the researchers found that in living mice, the dopamine-producing neurons are capable of triggering nerve impulses even when they are deprived of dopamine. Scientists are trying to find how even with insufficient amount of the neurotransmitter like the dopamine, the cells are able to outburst and transmitting impulse. Another unanswered question is that, what could the medicine for the patients who have lost most of their dopamine-producing cells? The relevance of this question is because the L-dopa just increases the production of dopamine in the cells, which are healthy and are producing dopamine. So, the cure for the situation mentioned in the question remains unanswered.

Paul Greengard- Nobel Prize for Discovery of how dopamine and other neurotransmitters work in the nervous system.

Literature Review And Discovery

Prior to Paul Greengard's research, less was known about the function of different receptors and organisation of various dopamine pathways which produce normal behavior and exhibit disruption in case of psychiatry disorders. Little was known about utility of slow signal transduction pathways and its relationship with fast signal transduction. Also, brain signalling was exclusively electrical and not chemical. This theory was in sharp contrast to the laboratory results on why adenylyl cyclase took many seconds to minutes to generate electric signals.

Carlsson in late 1950s identified dopamine as one of the potential neurotransmitters. It was also associated with Parkinson disease. Where low dopamine concentrations were observed in the putamen and caudate nucleus. Therefore, levodopa was used to treat Parkinson which is a metabolic precursor of dopamine. Two groups of receptors for dopamine were identified. First, have the ability to stimulate cAMP receptors and exert their effect through a cascade of a biochemical reaction. These slow transmission signals were utilized in neuro-modulation and long-term regulation. The second group of receptor-stimulated and inhibit the activity of adenylyl cyclase and were found in the neostriatum. This group was further divided into two classes, D1 And D2. Later it was shown there were 5 receptors, two D1 like receptors (D1, D5) and three D2 like receptors (D2, D3, D4). Progress in cellular electrophysiology and behavioural studies were crucial to determining the function of dopamine neurons. In monkeys, dopamine frequency was increased by unexpected reward and decreased in absence of expected awards. This finding contributed to the role of dopamine in conditional learning. It was found that dopamine receptors comprised of 7 trans-membrane domain receptors and GTP binding protein receptors. D1 receptors raised cAMP levels and thereby activating cAMP-dependent protein kinase, called PKA. D2 receptors are coupled with G proteins to decrease cAMP levels and permeability of potassium ion channels.

The experiments conducted to define dopamine mechanism involved striatum. Many efforts were done to determine protein phosphorylated by PKA and its regulation mechanism, in vitro. The method used involved antibodies which recognized a protein only when it is phosphorylated at a precise location on specific amino acid residue. These antibodies were obtained by immunization of rodents against a short phosphorylated polypeptide corresponding to a specific site. Affinity purification was performed to select antibodies with a phosphorylating agent. These antibodies allow the recognition of phosphorylated protein with the help of immunoblot or by immunofluorescence.

The above-mentioned experiment methods revealed various phosphorylated proteins. It was revealed that dopamine controlled the activity of glutamate receptors which mediate corticostriatal neurotransmission. It was done by PKA phosphorylating of AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) and NMDA (N-methyl-D-aspartate) glutamate receptors. Dopamine's phosphorylation also maintained the opening and closing of voltage-gated ion channels like sodium and potassium. Transcription factors were also discovered which imparted dopamine capability to make a long-lasting modification of synaptic transmission. Various regulatory proteins for signal cascade were also discovered. The major regulatory protein identified was DARPP-32. This protein when phosphorylated on threonine 34 by PKA, in response to D1 receptor stimulation, acts as an inhibitor of protein phosphatase-1. The significance of this inhibition was studied in a population of mutant mice lacking DARPP-32. These mice appeared normal in standard laboratory housing conditions but had a very abnormal response to cocaine and other dopamine triggering cocaine agents. DARPP-32 also phosphorylates CDK-5 to turn it into a PKA inhibitor. It showed that DARPP-32 acts as a switch which shuffles between amplification and inhibition regulation mechanisms in the cAMP pathway. This regulation of CDK5 by DARPP-32 was considered significant to understand the adaptive response of striatal neurons to the repetitive stimulation of dopamine receptors by cocaine. This indicated that DRPP-32 is just not a limited part in the cAMP pathway but plays a crucial role in many signaling pathways and their action in striatal neurons. The response modulation and synaptic plasticity alteration by dopamine account for its role in various psychiatric disorders such as Parkinson disease, schizophrenia, attention-deficit/hyperactivity disorder etc. This happens by various kinds of alterations causing atomic-site dysfunction and hence, different clinical manifestations. Understanding different intracellular signalling pathways, the action of neurotransmitters is essential to develop therapeutic and pharmacological agents. At present, these studies are constrained to humans and novel methods are required to study these processes in vivo using non-invasive methods.

Current Status And Future Trends

Paul Greengard study was revolutionary in the sense that he was able to elucidate exactly what happens when a neurotransmitter touches base with its target neuron. Greengard's hypothesis that neurotransmitters can act similar to hormones. This was proved by the presence of neurotransmitter enzymes and a chain of biochemical events. This led to a shift in the scientific community's view that the brain demonstrates chemical activity, and not only electrical activity. He was also able to show that slow signal transduction was far more common than fast signal transduction. DRPP-32 discovered by him has found its application in a dozen neurotransmission pathways. Finally, his demonstrations of causative connection between dysfunctions in dopaminergic pathways have led to dopamine targeted treatment for many psychiatry disorders along with providing insights into the brain's response to addictive substances.

A lot is known about D1 and D2 receptors of dopamine, but lot less about the other three, although these information are expected to emerge with time. For example, it is proven that D5 receptors is involved in rewarding aspects of sexual behaviour in mice. This new research can further contribute in the present understanding of neuron transmission and modulation. Another ongoing research is whether reward stimulation can take place in absence of dopamine. The evidences have proven that mice lacking tyrosine hydrolase and are unable to perform dopamine synthesis still show sucrose preference, which is a reward behaviour. Progress is also expected to understand the link between dopamine receptor occupation and altered gene expression. There are the evidences that it may involve *β*-arrestin-2 dependent mechanisms instead of standard adenylyl cyclase and CREB phosphorylation. Research is also underway on alternative of the D2 receptor which is said to be action through prostate apoptosis response-4. Since now, the focus was only on study of properties and dopamine mechanisms in isolation. However, neuronal organisation of cortical areas with links in the subcortical regions have made it necessary to conduct dopamine related studies in an integrated manner. Dopamine neurons constitute only 1% of the total brain neurons, but its profound effect in behaviour suggests that dopamine relays integrate information with regard to a specific biological stimuli. Therefore, dopamine research in an integrated manner and resulting therapeutic treatments for psychiatric disorders is something the future holds.

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