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"That which is eternally incomprehensible to us in Nature is her comprehensibility"
EINSTEIN

Introduction

The advances which in these two past decades have transformed the field of neurobiology in one of the most rapidly moving frontiers in life sciences, would make it most difficult to present, even if in a summary fashion, the main lines of investigations which have been pursued during this period. The impossibility of assolving to such a task, is taken as justification for considering only one restricted area of this field dealing with the monoaminergic central and peripheral neurons. These cells, which have of recent come to the forefront of neurobiology for the manifold and most important

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functions which they serve in the central nervous system, came already to the attention of the neurophysiologist in the first half of the century, when they revealed the humoral basis of the transmission of the nerve impulse. Without the adrenergic sympathetic and the cholinergic parasympathetic nerve cells, it is doubtful whether the chemical nature of the neurotransmitters and the subsequent identification of some of them, would have ever been achieved. And yet, even if it was soon to be proved that intracentral neurons communicate by releasing humoral agents, in the same way as the peripherally located adrenergic and cholinergic nerve cells, still the concept prevailed that these peripheral nerve cell populations form a class apart from those of other neurons, being as they are, excluded from the main stream and endowed with what seemed to be a sort of ancillary role with respect to that of the centrally located neurons. Hence, only a decade ago, the neurobiologist would have been reluctant to accept the adrenergic sympathetic nerve cell as a valid model of neurons responsible for higher brain functions.

The discovery that nerve cell populations in the central nervous system, share many properties in common with the sympathetic adrenergic neurons, and at the same time play a fundamental role in the structural and functional organization of the brain, opened what can be defined as the golden era in neurobiological sciences. The heavy investment in this past decade in the study of monoamine-containing neurons, and of the role which they play in normal and abnormal brain functions, gives impressive evidence for these new developments, which in turn resulted in the revival of interest in the sympathetic adrenergic neuron for the vicarious and most serviceable role that this cell can play as a model system of the central much less approachable monoaminergic neurons.

In the following pages we shall consider at first some lines of investigation which led to the discovery of the role of dopaminergic neurons in diversified activities of basal ganglia and in the dramatic restoration of some neurological and mental

disorders, obtained by supplying the precursor of the neurotransmitter or by interfering with its binding to its specific receptor.

In the second part we shall review other studies which gave evidence for the outstanding role of a protein molecule, normally present in vertebrate tissues and organic fluids, in controlling growth and differentiation of the sympathetic adrenergic nerve cell.

a) *The adrenergic neuron*

The sympathetic nerve cells which were to play an ever-increasing role in basic research on metabolic processes related to the synthesis, release and degradation of the neurotransmitter, as well as in providing a sort of Ariadne thread to gain access inside the labyrintic complexity of the vertebrate nervous system, and uncover some of its functions, came first to the attention of the neurologist toward the end of the past century. We are indebted to an anatomist, W. H. GASKELL and to a pharmacologist, J. N. LANGLEY for the first accurate structural and physio-pharmacological analysis of the two slender chains of nerve cells positioned at the sides of the neural tube and connected with the central nervous system (CNS) through the agency of nerve fibers emerging with the motor roots of the spinal cord [1, 2].

The function of this system which retains some autonomy with respect to the CNS and for this reason became known as the autonomic or sympathetic nervous system, consists, as all too well known, in the regulation of the contractility of blood vessels, smooth muscles in peripheral tissues and organs, heart beating and hair erection. In the first decade of this century the similarity between the effects called forth by the stimulation of the sympathetic ganglia and of the hormone produced by adrenal medulla, adrenaline, suggested the hypothesis that sympathetic nerve fibers release a neurohormone closely related

to adrenaline [3]. This remarkably correct guess had however the fate of all premature discoveries which do not fit in preconceived schemes and do not conform with accepted dogmas; it was rejected by leading authorities in this field and first of all, by the world expert on sympathetic nerve cell function and ELLIOTT's tutor, J. N. LANGLEY. Thirthy years later, unequivocable evidence for the humoral nature of the sympathetic neurotransmitter was provided by H. DALE [4], while the same evidence had already been given a decade earlier by O. LOEWI with the discovery of the "Vagusstoff" as the neurotransmitter of the parasympathetic nerves [5].

The term "adrenergic" in replacement of that of "sympathetic" was proposed by H. DALE in 1933 "to assist clear thinking, without committing us to precise chemical identification, which may be long in coming" [4]. The hypothesis of the humoral nature of the neurotransmitter as well as the new terminology, were this time received favorably and immediately adopted in the literature. The caution of DALE in suggesting the rather general term "adrenergic" proved to be wise since thirteen years later, U.S. VON EULER showed that noradrenaline rather than adrenaline is the humoral transmitter released by sympathetic nerve fibers [6]. This discovery in turn sparked a series of brilliant investigations that in a decade period resulted in the identification of all enzymes involved in the synthesis of noradrenaline (NA), in the discovery of its mechanism of storage, release and enzymatic degradation or re-uptake by adrenergic fibers and the activation of post-synaptic receptors [7, 8, 9]. The elucidation of the metabolic pathway of NA and of the subsequent processes of storage, release, degradation or re-uptake, opened the way to the study of pharmacological agents which prevent or enhance each one of these processes as well as the binding or conversely the blocking of specific receptors [10]. The subsequent discovery that NA analogs share with NA the property of being selectively uptaken by the same nerve endings but, upon release, fail to activate the receptors [11], offered endless possibilities of

interfering with the function of this remarkably tolerant and cooperative nerve cell. The clinical implications of these findings on the control of the sympathetic function which plays a prominent role in the regulation of blood flow, were an additional incentive to develop an ever-growing list of compounds that act at different steps of the synthesis, release and binding of NA to its receptors, and in this way proved to be of great value in the treatment of clinical disorders such as hypertension [12, 13].

b) *The central monoaminergic neuron*

While these studies were in progress, two discoveries, a few years distant from each other, succeeded in effacing the psychological barrier which had until then practically prevented "cross-talk" between students of the two main division of the vertebrate nervous system: the cerebrospinal axis and the peripheral autonomic ganglia.

In 1954 M. VOGT discovered that NA is present at different concentrations in different sections of the CNS and she submitted the hypothesis that some neuronal cell populations in the brain and spinal cord synthetize and release this monoamine in the same way as the peripheral adrenergic sympathetic neurons [14].

In 1962, Swedish investigators devised a new histofluorescent technique which made possible the visualization of biogenic amine containing nerve cells, in the peripheral and central nervous system [15].

In the same way as the discovery of the affinity of nerve cells for silver salts a century earlier, had revealed the tremendous variety in size, shape and architectural organization of nerve cell populations embedded in the dense matrix of the nervous system, and made possible to trace the wiring circuits among different centers, now this technique which takes advantage of the fluorescence developed by biogenic amines

upon reaction with formaldeyde vapors, disclosed the presence, among the 15 billions of nerve cells which build the central nervous system of higher vertebrates, of a few thousand nerve cells endowed with the property of synthetizing and releasing two classes of biogenic amines: the catecholamines and the indolamines. Three types of catecholamine nerve cells are found in the CNS of vertebrates: dopaminergic, noradrenergic and adrenergic. While they all contain a catechol and an amine group and utilize the same metabolic pathway, leading from tyrosine to dopamine, some nerve cells located in higher brain centers, release dopamine; others assembled in small nuclei or scattered as isolated units in the brain stem synthetize and release respectively noradrenaline or its methylated derivative, adrenaline. Indolamine cells also known as serotoninergic cells, synthetize serotonin from tryptophan. They are found in the brain stem and are easily distinguishable at the ultraviolet microscope from catecholamine nerve cells thanks to the yellow rather than green fluorescence which they emit upon reaction with formaldehyde [16].

Mapping of fluorescent nerve cells and of their axons assembled in bundles or loosely distributed as individual fibers among the vast majority of non-fluorescent fibers which run in the CNS, brought to light three additional features of these mono-aminergic system: a) the paucity in number of catechol- and indole-amine cells is compensated by an extraordinary diffuse branching of their axons which in this way come in synaptic or non-synaptic contact with a very large number of sub-cortical and cortical brain areas. b) Catecholaminergic and indoleaminergic systems originate in nuclei which belong to the extrapyramidal system, namely to the phylogenetically oldest motor system which is present with only slight modifications in the Central Nervous System of all vertebrate, man included. c) Dopaminergic cells located in a large nucleus known as the substantia nigra in the mesencephalon and in smaller adjacent cell aggregates, establish connection with paleo-cartical and sub-cortical nuclei of the limbic system, also known as the

circuit of emotion, in view of the dramatic changes in behaviour which follow to lesions or activation of one or the other of these centers in mammals and in man.

These discoveries paved the way to the study of the effects of pharmacological agents which were shown to interfere with the function of peripheral adrenergic neuron, by enhancing or blocking the release of the neurotransmitter, noradrenaline.

The results of these investigations which took their start in the early sixties and moved at an increasingly faster pace in the following decade up to the present day, triggered what has been defined as a revolution in psychiatry as well as in the treatment of some disorders of motion attributed to disfunction of the extrapyramidal system. Thus a field which has been the most stagnant area of biological sciences for this last century and which seemed to be hopelessly out of reach to investigators, such as the understandig of the causes and restoring of functions dramatically altered in some brain illness, became all of a sudden one of the most rapidly moving frontiers in neurobiology. These progresses so much above the expectations of investigators only one decade ago, have been realized thanks to the extraordinary advances in our knowledge of the peripheral adrenergic neuron which served as a model of the central neuron endowed with the same property of synthetizing and releasing biogenic amines [17].

Since it would not be possible to review, the new developments which took place simultaneously at the molecular, biochemical, structural and behavioural levels in this area, I shall only mention the two most significant achievements in the elucidation of two apparently unrelated disorders such as the Parkinson disease and schizophrenia. The former is a neurological disease characterized by motor disfunction of the extrapyramidal type. Its cardinal symptoms are: akinesia or inability to initiate movements, tremor and rigidity. Histopathological studies gave evidence for a severe loss of neurons of the compact layer of the mesencephalic nucleus known as the substantia nigra in view of the brown color of its cells which impressed

the earlier investigators of brain centers. Studies at the ultraviolet microscope showed that the nerve cells of this nucleus stained according to the FALCK-HILLARP technique [15] emit a green fluorescent light characterictic of the catecholaminergic cells. Biochemical studies identified in dopamine, the biogenic amine present in these neurons and in their axons which end in synaptic contact with different nuclei of the basal ganglia of the striatum complex, one of the main station of the extrapyramidal system. The destruction of the dopaminergic cells of the substantia nigra and of their axons and the subsequent depletion of dopamine in the striatum, may result from a large number of unrelated causes such as senile arteriosclerosis, postencephalitic viral infection, chemical cytotoxic effects produced by manganese or by some pharmacological agents which selectively concentrate in these nerve cells. The finding by A. CARLSSON that reserpine, a powerful catecholamine depleting agent produced a Parkinson-like tremor in animals and that the administration of l-dopa a dopamine precursor which passes the blood-brain barrier reversed the tremor [18] focussed the attention on this biogenic amine for its possible role in the normal function of the striatum and in particular on one of its components known as the caudate nucleus. A few years later another pharmacologist O. HORNYKIEVICZ found that in patients who had died of Parkinson disease, dopamine was virtually absent in the nucleus caudate [20, 21]. These observations prompted still another pharmacologist, G.C. COTZIAS to administer l-dopa to patients suffering of the Parkinson disease [22]. The dramatic improvement of this, up to that time, unmanageable disorder, offered an invaluable help to the ill and at the same time represented the first successful therapy replacement instance of a severe neurological disorder based on the biochemical identification of its causative agent.

At variance with the Parkinson disease, schizophrenia is, above all, a disorder of thought and of the most subtle aspects of interpersonal relations [23]. As such, it is an illness which cannot take advantage of animals as experimental models, in

view of our inability to communicate with them through that unique communication system devised by man which is the use of language.

The discoveries in 1935 [24] that high doses of a powerful catecholamine releasing agent, amphetamine, produces in man a syndrome which mimics to a remarkable degree the paranoic schizophrenic syndrome and in 1957 [25] of another pharmacological agent, chloropromazine belonging to the class of phenothiazines, which remarkably improves the schizophrenic patient but at the same time produces extrapyramidal side-effects of the Parkinson type, were the starting points of investigations on the possible role of dopamine and other biogenic amines in the insurgence of this psychotic illness. In this last decade suggestive evidence has been obtained in favor of the hypothesis that schizophrenia is a genetically determined metabolic disorder of the dopamine mesolimbic system linked to emotional behaviour. The dopamine theory of schizophrenia has gained strong support from studies on the molecular basis on antipsychotic drug action analyzed at the biochemical level in brain centers [28] as well as in the superior cervical ganglion where dopaminergic interneurons provided an invaluable model system to examine the effects of drugs affecting behaviour, on these dopamine containing neurons and on the release of the neurotransmitter on the postsynaptic sympathetic neuron [29]. The concept was developed that antipsychotic drugs owe their pharmacological effect to their property of blocking dopamine receptors [30]. In so doing, they counteract the hyperactivity of dopamine mesolimbic centers which results from inborn defects or from drugs such as amphetamine which enhance the release of dopamine and produce schizophrenia-like mental aberrations.

In closing this very succint report on the theory now in favor on the role of dopaminergic neurons in this most severe psychotic disorder, it should be kept in mind that one should accept it for its heuristic value in promoting further investigations along this line which may bring additional information in

favor or againts this hypothesis. As stated by FRIEDHOFF: "All scientific exploration requires reductionism in order to make a problem more manageable" [31]. In no instance this is more appropriate than in the case of the study of brain functions where the staggering complexity of neuronal circuits and of their interactions has until now prevented any even preliminary approach to the problem.

To the outsider and to men in general, who ever since the dawn of civilization have been frightened by mental illness and have conceived a resentment akin to hate for the ill who has been confined in mental institutions where the last remnants of his personality and human dignity were thorn apart by the same effect of the confinement and of the restraining devices, the most significant achievements of these studies is to have developed drugs which remarkably improve the clinical syndrome and restore mental functions disrupted by the disease.

To the psychologist as well as to the student of brain normal and abnormal activity, a no less valuable accomplishment is to have provided strong evidence in favor of the hypothesis of a biochemical inborn disorder and to have identified in the dopaminergic nerve cells of the mesolimbic system, the cells which are likely to be implicated in the insurgence of the abnormal behaviour.

To the neurobiologist who is mainly concerned with the study of nerve cell function, it is motive of great pride and encouragement to have for the first time been able to gain access inside the tremendous complexity of the brain and uncovered some of the processes which operate at the cellular level in motor as well as mental functions.

c) *The sympathetic adrenergic nerve cell as a model system of nerve cell growth and differentiation*

While studies on the function of nerve cells synthetizing and releasing biogenic amines were in progress, the sympathetic

adrenergic neuron came again to the forefront of research in an entirely different area of neurobiology dealing with the role of intrinsic or genetic, and of extrinsic or environmental factors in growth and differentiation of nerve cells.

In the course of investigations on the role played by end organs and peripheral tissues in the development of primary motor and sensory nerve cell populations in the chick embryo, it was found that sensory [32] and sympathetic ganglia undergo a marked volume increase when some malignant mouse tumors were implanted into the body wall or onto the chorio-allantoic membrane of the embryo [33]. In the case of tumor transplantation onto the chorio-allantoic membrane, the tumoral and chick tissues share the circulation but no direct contact is established between neoplastic and embryonic tissues. The size increase of ganglia in embryos bearing chorio-allantoic tumor transplants was therefore taken as indicative of the release by the tumors of a humoral factor that selectively enhances growth and differentiative processes of sensory and sympathetic embryonic nerve cells [34]. In 1954 this factor on account of its biological activity was named the Nerve Growth Factor or, more simply, the NGF [35]. Before reporting on recent developments in this field I shall briefly review some of the previous work which provided the basis for subsequent studies.

An in-vitro assay developed soon after the discovery of the growth promoting activity of some mouse sarcomas in the chick embryo made possible the rapid screening of normal and neoplastic tissues for their potential nerve growth promoting effects on the same nerve cells which are receptive in vivo to the NGF [36]. This test consists in explanting in tissue culture with the hanging drop technique, embryonic sensory or sympathetic ganglia in proximity of the tissues to be examined as putative NGF sources. Tissues extracts or organic fluids were also assayed by adding them at various dilutions to the culture medium. The NGF effect elicited by tissues or fluids consists in the formation of a dense fibrillar halo of nerve

fibers around sensory or sympathetic embryonic ganglia in a 10-24 hour period. In the absence of NGF only few nerve fibers grow out from the ganglia and branch at random in the medium. This rapid and most reliable assay made possible the identification of the tumoral NGF in a protein molecule present in the tumor extract [35].

The same technique revealed a few years later the presence of a nerve growth promoting activity in two different and unrelated sources: the snake venom and the mouse salivary glands [37, 38]. The isolation from the snake venom and from the salivary glands of two protein molecules endowed with physico-chemical properties remarkably similar and biological activity identical with those of the tumoral NGF, and the finding that in both materials the NGF is present at a concentration 5- to 10,000 time higher than in mouse tumors, diverted the attention from the tumors to the snake venom and then to the salivary glands which represent a far more manageable and convenient source of NGF than the snake venom. Ever since 1958 almost all studies on NGF in our and other laboratories which became interested in this remarkable growth promoting protein, centered on the salivary NGF. Here I shall review only the most relevant features of this factor and of its biological activity.

The salivary NGF first isolated and identified by S. COHEN in a protein molecule of estimated molecular weight of 44,000 [39] was the object of extensive studies in subsequent years. In 1969 BOCCHINI and P.U. ANGELETTI isolated a biologically active moiety with a sedimentation coefficient of 2.5 S and an apparent m.w. of 30,000 [40]. Studies by ZANINI *et al.* [41] indicated that the NGF could be separated into biologically active fractions of 28,000 and 14,000 m.w. In 1971 R.H. ANGELETTI and R.A. BRADSHAW [42] determined the primary structure of the salivary NGF and established that it consists of two identical sub-units held together by non-covalent bonds. Each subunit is composed of 118 amino-acids with a resultant m.w. of 13,259, possessing amino ter-

minal serine and carboxyl terminal arginine. The native protein has a molecular weight of 26,518 and an isoelectric point of 9.3. Three disulfide bonds (a feature common to most "exportation" protein molecules) impart a particularly rigid and resistant nature to the NGF moiety as indicated by its resistance to enzymatic, chemical and heat denaturation [43]. In 1975 A. WLODAWER *et al.* succeeded in crystallizing the NGF, a feat which brings within reach the goal of uncovering the tertiary structure of NGF and of exploring at the molecular level its interaction with its target cells [44].

Studies in vivo consisted in the daily subcutaneous injections of 10 μg/gr NGF of body weight in newborn and adult rodents. In the former the NGF produces in a ten-day period a volume increase of sympathetic ganglia of 10-12 fold that of controls. This effect is due to increase in number and size of individual neurons. In the fully developed animal the response evoked by NGF is a volume increase of two-three times that of controls. This marked difference between newborn and adult rodents is due to the lack in the adult animal of the mitotic effect which is instead prominent in ganglia in the first post-natal week [45]. The hypertrophic and hyperplastic ganglia hyperneurotize peripheral organs and tissues. Studies at the U.V. microscope with the histochemical fluorescent technique showed that the peripheral adrenergic plexus is much more dense and more intensely fluorescent than in controls. At the ultrastructural level the most outstanding as well as the earliest NGF effect in nerve cells of newborn or adult animals is a massive increase in neurotubules and neurofilaments in the cell perikarya and in their axons [46].

At the metabolic level the NGF effects do not substantially differ from those produced by other growth factors and hormones on their target cells. The response conforms with the definition of "positive pleiotypic response" given by A. HERSHKO *et al.* in their analysis of the metabolic effects evoked in the receptive cells by a number of seemingly unrelated

biological factors [47]. It is of considerable interest to note that in the sympathetic immature and fully developed nerve cells, the NGF also markedly enhances the synthesis of the specific neurotransmitter NA, as indicated by studies on the specific activity of the two key enzymes of the metabolic pathway of noradrenaline, thyrosine hydroxylase and dopamine β-hydroxylase. Both enzmes undergo significant increase in sympathetic ganglia of NGF treated mice and rats [48].

The results of the studies briefly summarized here brought to the fore the hitherto unsuspected capacity of the sympathetic adrenergic neuron to undergo a dramatic size increase when a protein molecule which is normally present in blood and body fluids is made available in quantities far exceeding the normal level. The enhanced synthesis of the neurotransmitter gives additional evidence for the specificity of this growth effect which promotes not only cell growth but also the metabolic pathway related to the cell function.

c) *Growth suppression and destruction of the sympathetic adrenergic neuron*

The discovery in 1959 that a specific antiserum to NGF produces the destruction of the immature sympathetic nerve cells, an effect which became known as immunosympathectomy [39, 49], further stressed the unique role of this protein molecule in the life of its target cell. More recent studies showed that the same immature neuron undergoes regressive changes, leading to its death, when pharmacological agents which gain access to the cell through the amine pump, accumulate inside the cell where they exhert their severe cytotoxic effects. The two agents which produce this effect, are guanethidine and 6-hydroxydopamine [50, 51]. Guanethidine was introduced in the medical treatment of hypertension in view of its action of lowering the arterial pressure in animals and man. Upon

uptake by the nerve fiber terminals, guanethidine gains access to the perikaryon where it exherts its damaging effects on the cell metabolism and blocks the synthesis of the neurotransmitter. Prolonged treatment causes irreversible changes also in the fully differentiated cell resulting not only in the suppression of the cell specific function but also in irreversible inhibition of the cell metabolic processes which causes its death [52]. At the ultrastructural level these alterations consist of the total disruption of the mitochondria followed by degenerative changes in the cytoplasmic compartment and then in the nucleus [51]. 6-hydroxydopamine (6-0HDA), a dopamine derivative produces depletion of NA in hearts of mice and rats. The duration of this effect lasts 6-8 weeks, namely a much longer period than that of other pharmacological agents such as guanethidine and brethylium. Ultrastructural studies by TRANZER and THOENEN [53] elucidated the cause of this long lasting NA depletion. The dopamine derivative accumulates selectively in the synaptic vescicles of noradrenergic nerve endings where the auto-oxidation products of 6-OHDA cause the total destruction of these vescicles, thus preventing the transmission of the neurotransmitter to the post-synaptic cells. When injected in newborn rodents or other mammals 6-OHDA produces the total destruction of the sympathetic nerve cell population. At variance with the effects produced by guanethidine and other-like compounds which interfere with the cell metabolic processes, there is no evidence of degenerative lesions in the cytoplasmic or nuclear cell compartments. The nerve cell death which follows to this treatment in newborn rodents is apparently due to the "chemical transection" operated by 6-OHDA and suppression of the input-output traffic at the nerve fiber terminal ending at a most vulnerable period of its growth and differentiation. In favor of this hypothesis is the finding that surgical axotomy performed at the same development period also produces destruction of the sympathetic nerve cell population of surgically transected ganglia [54].

d) *New developments in the study of the Nerve Growth Factor*

Recent studies on the NGF-target cell interaction added further evidence for the outstanding role played by this protein molecule in the life of the sympathetic neuron. The results of these investigations will be only briefly summarized here.

Both the noxious effects of guanethidine and 6-hydroxydopamine (6-OHDA) on the immature sympathetic nerve cells, are obliterated by the simultaneous injection of NGF. The end results of the combined NGF and guanethidine or NGF and 6-OHDA treatments are however markedly different.

Administration of the guanidium adrenergic blocking agent, guanethidine, causes permanent destruction of immature sympathetic nerve cells by a cytotoxic mechanism involving inhibition of mitochondrial respiration. The ability of NGF to prevent the destruction of sympathetic ganglia, does not appear to result from an inhibition of guanethidine accumulation in the sympathetic nerve cells, as indicated by the finding that discontinuation of the NGF treatment results in the destruction of the sympathetic nerve cells due to the ability of the accumulated guanethidine to produce its toxic action once it is no more counteracted by NGF [55]. It remains to be established how NGF prevents the cytotoxic lethal effect of guanethidine.

A much more dramatic effect is produced by the simultaneous administration of NGF and 6-OHDA. Sympathetic ganglia of neonatal rodents are not only spared the destructive lesions produced by 6-OHDA when treated simultaneously with NGF, but undergo a volume increase much more impressive than when treated with NGF alone. This paradoxical effect is due to hyperplastic and hypertrophic effects on the neuronal cell population and to the overproduction of collateral fibers by the adrenergic axons. This latter effect is due to the cytotoxic effects elicited by the dopamine derivative on the adrenergic terminals and their chemical disconnection from end organs. Sympathetic nerve cells isolated from end organs

by the accumulation of 6-OHDA in the adrenergic endings but geared to a higher than normal metabolic activity by the daily supply of large quantities of NGF, synthetize an excessive amount of axonal material which is channelled in the collateral fibers inside and around the ganglion [56, 57, 58].

e) *The NGF and the central monoaminergic neuron*

The markedly similar structural and functional properties of the sympathetic adrenergic neurons and of the central nerve cells located in the brain stem in the nuclei of the loci coerulei and other small noradrenergic cell aggregates, raised the question whether these cells are receptive to the NGF as the peripheral adrenergic sympathetic nerve cells. Several studies by Swedish authors [59, 60, 61] favored this view. More recent investigations in our and other laboratories (unpublished results) did not confirm these early reports and the concept now prevails that the central monoaminergic (catecholaminergic as well as indoleaminergic) neurons do not respond to the NGF nor are destroyed by specific antibodies to this protein molecule. These negative findings leave however open the possibility that growth and differentiation of the central monoaminergic neurons are under the control of like or unlike protein factors released by other cells. A possible candidate for such a role is a "growth factor" isolated from glial cells in the central nervous system and from some glial neoplastic cell lines [62, 63].

Concluding remarks

In commenting on the statement attributed to EINSTEIN that "which is eternally incomprehensible to us in Nature is her comprehensibility", R. HOUNWINK writes, "however complex her laws may seem to us, Nature conducts her affairs according

to simple principles, and all her creations — whether base matter or the stuff of life — are cast in simple moulds [64]".

We attempted to illustrated this viewpoint which we share, in this short essay.

The tragedy of mental illnesses which, ever since the dawn of civilization have plagued mankind and have been considered as the worse evil which can afflict human beings, is now, for the first time, visualized in terms of disfunction of specific brain circuits rather than aggravated by the emotional and irrational reaction which for centuries have indicated in the confinement and harsh punishment, the only possible treatment of the mentally ill. Such most welcome change in attitude must be credited to the success obtained in unravelling some brain circuits and understanding their mechanism of action. This became possible thanks to their similarity with peripheral circuits which lent themselves to structural and biochemical analysis in view of their favorable position as well as their simple and almost diagrammatic construction plan. It became in this way possible to gain information on neural systems which had until now defied all attempts of a direct study, due to their staggering complexity, as well as to their location in the dense matrix of the central nervous system.

The results achieved in less than two decades, are already of such magnitude, particularly if evaluated for their prospective rather than actual value, and if compared with the previous status of our knowledge, as to raise hopes in future even more significant developments, in the fields of nerve cell differentiation and of the structure and function of neuronal circuits. Thus, the most cherished ambitions of man, to understand the mechanism of action of these circuits and to find an explanation and possible means of repairing disfunctions, are now approaching the time of their realization.

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BIBLIOGRAPHY

[1] GASKELL W.H., *On the structure, distribution and function of the nerves which innervate the visceral and vascular system.* « J. Physiol. », *7*, 1 (1886).

[2] LANGLEY J.N., *The autonomic Nervous System.* Part. 7, 80 pp., Cambridge, W. Heffler and Sons (1921).

[3] ELLIOTT T.R., *The action of adrenalin.* « J. Physiol. », *32*, 401 (1905).

[4] DALE H., *Nomenclature of fibres in the autonomic system and their effects.* « J. Physiol. » (Lond.), *80*, 10-11P (1933).

[5] LOEWI O., *Ueber humorale Uebertragbarkeit der Herznervenwirkung.* « Naturwissenschaften », *10*, 52-55 (1922).

[6] V. EULER U.S., *A specific sympathomimetic ergone in adrenergic nerve fibres (sympathin) and its relations to adrenaline and noradrenaline.* « Acta physiol. scand. », *12*, 73-97 (1946).

[7] UDENFRIEND S., *Biosynthesis of the sympathetic neurotransmitter, Norepinephrine.* « The Harvey Lectures, S. », *60*, 57-83 (1966).

[8] IVERSEN L.L., *The uptake and storage of Noradrenaline in Sympathetic Nerves.* Cambridge University Press (1967).

[9] AXELROD J., *Noradrenaline: Fate and control of its biosynthesis.* « Science », *173*, 598-606 (1971).

[10] BURNSTOCK G. and COSTA M., *Adrenergic neurons: their organization and development in the peripheral nervous system.* Chapman and Hall, London (1975).

[11] KOPIN I.J., *False adrenergic transmitters.* « Ann. Rev. Pharmacol. », *8*, 377-394 (1968).

[12] MALFORMS T. and THOENEN H. eds., *6-Hydroxydopamine and Catecholamine Neurons.* North-Holland Publishing C. (1971).

[13] USDIN E. and SNYDER S.H. eds., *Frontiers in Catecholamine Research.* Proc. of the Third Intern. Catecholamine Symposium, Pergamon Press (1973).

[14] VOGT M., *The concentration of sympathin in different parts of the central nervous system under normal conditions and after administration of drugs.* « J. Physiol. » (Lond.), *123*, 451-481 (1954).

[15] FALCK B., HILLARP N.A., THIEME G. and THORP A., *Fluorescence of catecholamines and related compounds condensed with formaldeyde.* « J. Histochem. Cytochem. », *10*, 348-354 (1962).

[16] COOPER J. R., BLOOM F. E. and ROTH R. H., *The Biochemical Basis of Neuropharmacology.* Oxford University Press, 2nd ed. (1974).

[17] BLASCHKO H. and MUSCHOLL E. eds., *Catecholamines.* Springer Verlag, Berlin (1972).

[18] CARLSSON A., LINDQVIST M., MAGNUSSON T. and WALDECK B., *On the presence of 3-hydroxytyramine in Brain.* « Science », 127-471 (1958).

[19] HORNYKIEVICZ O., *Zur Frage des Verlaus Dopaminergic Neurone im Gehirn des Menschen.* « Wien Klin. Wschr », *76*, 834-835 (1964).

[20] HORNYKIEVICZ O., *Dopamine* (3-hydroxytyramine) *and brain function.* « Pharmacol. Rev. », *18*, 925-964 (1966).

[21] HOENYKIEVICZ O., *Parkinson's disease: from brain homogenate to treatment.* Symposium on: Contributions of Neurochemistry and Psychiatry, 3rd Meeting of the Amer. Soc. for Neurochemistry, Seattle (1972).

[22] COTZIAS G. C., *Levodopa, Manganese and Degeneration of the Brain.* « The Harvey Lecture S. », *68*, 115-147 (1974).

[23] MATTHYSSE S., *Antipsychotic drug actions: a clue to neuropathology of schizophrenia?* « Fed. Proceed. », *32*, 202-205 (1973).

[24] PRINZMENTAL M. and BLOOMBERG W., *Use of Benzedrine for treatment of narcolepsy.* « J. Am. Med. Assoc. », *105*, 2051.

[25] SNYDER S. H., *Catecholamines as Mediators of Drug Effects in Schizophrenia.* In: « The Neurosciences Third Study Program », (Schmitt F. O. and Worden F. G. eds.), 721-732 (1974).

[26] MATTHYSSE S., *Schizophrenia: Relationship to Dopamine Transmissions, Motor Control, and Feature Extraction.* In: « The Neurosciences Third Study Program », (Schmitt F. O. and Worden F. G. eds.), 733-740 (1974).

[27] HOLLISTER L. E., *Chemotherapy of schizophrenia.* In: « Brain Chemistry and mental Disease », (eds. Ho B. T. and McIsaac W. M.), Plenum Press, 303-317 (1971).

[28] KETY S. S., *Biochemical theories of schizophrenia.* Part. I of a two part critical review of current theories and of the evidence used to support them. « Science », *129*, 1528-1532 (1959).

[29] GREENGARD P., *Possible role for cyclic nucleotides and phosphorylated membrane proteins in postsynaptic actions of neurotransmitters.* « Nature », *260*, 101-108 (1976).

[30] SNYDER S. H. and BANERJEE S. P., *Amines in Schizophrenia.* In: « Frontiers in Catecholamine Research », (eds. Usdin E. and Snyder S. H.), Pergamon Press, 1133-38 (1973).

[31] FRIEDHOFF A. J., *Integration and Conclusions.* In: « Catecholamines and behaviour V. 2 Neuropsychopharmacology », (Friedhoff A. J. ed.), p. 216.

[32] BUEKER E. D., *Implantation of tumors in the limb field of the embryonic chick and developmental response of the lumbosacral nervous system.* « Anat. Record », *102*, 369-390 (1948).

[33] LEVI-MONTALCINI R., *Effects of mouse tumor transplantation on the nervous system.* « Ann. N. Y. Acad. Sci. », *55*, 330-343 (1952).

[34] LEVI-MONTALCINI R. and HAMBURGER V., *A diffusible agent of mouse sarcoma producing hyperplasia of sympathetic ganglia and hyperneurotization of the chick embryo.* « J. Exptl. Zool. », *123*, 233-288 (1953).

[35] COHEN S., LEVI-MONTALCINI R. and HAMBURGER V., *A Nerve-growth stimulating factor isolated from mouse sarcomas 37 and 180.* « Proc. Natl. Acad. Sci. U. S. », *40*, 1014-1018 (1954).

[36] LEVI-MONTALCINI R., MEYER H. and HAMBURGER V., *In vitro experiments on the effects of mouse sarcoma 180 and 37 on spinal and sympathetic ganglia of the chick embryo.* « Cancer Res. », *14*, 49-57 (1954).

[37] COHEN S. and LEVI-MONTALCINI R., *A nerve growth stimulating factor isolated from snake venom.* « Proc. Natl. Acad. Sci. U. S. », *42*, 571-574 (1956).

[38] LEVI-MONTALCINI R. and BOOKER B., *Excessive growth of the sympathetic ganglia evoked by a protein isolated from mouse salivary glands.* « Proc. Natl. Acad. Sci. U. S. », *42*, 373-384 (1960).

[39] COHEN S., *Purification of a nerve growth promoting protein from the mouse salivary gland and its neuro-cytotoxic antisierum.* « Proc. Natl. Acad. Sci. U. S. », *46*, 302-311 (1960).

[40] BOCCHINI V. and ANGELETTI P. U., *The nerve growth factor: purification as a* 30,000 *molecular weight protein.* « Proc. Natl. Acad. Sci. U. S. », *64*, 787-794 (1969).

[41] ZANINI A., ANGELETTI P. U. and LEVI-MONTALCIÑI R., *Immunochemical properties of the nerve growth factor.* « Proc. Natl. Acad. Sci. U. S. », *61*, 835-842 (1968).

[42] ANGELETTI R. H. and BRADSHAW R. A., *Nerve growth factor from mouse submaxillary gland: amino acid sequence.* « Proc. Natl. Acad. Sci. U. S. », *68*, 2417-2420 (1971).

[43] ANGELETTI R. H., BRADSHAW R. A. and WADE R. D., *Submit structure and amino acid composition of mouse submaxillary gland nerve growth factor.* « Biochemistry », *10*, 463-469 (1971).

[44] WLODAWER A., HODSON K. O. and SHOOTER E. M., *Crystallization of nerve growth factor from mouse submaxillary salivary glands.* « Proc. Natl. Acad. Sci. U. S. », *72*, 777-779 (1975).

[45] LEVI-MONTALCINI R., *The nerve growth factor: its mode of action on sensory and sympathetic nerve cells.* « Harvey Lectures Ser. », *60*, 217-259 (1966).

[46] LEVI-MONTALCINI R., CARAMIA F., LUSE S. A. and ANGELETTI P. U., *In vitro effects of the nerve growth factor on the fine structure of the sensory nerve cells.* « Brain Res. », *8*, 347-362 (1968).

[47] HERSHKO A., MAMONT P., SHIELDS R. and TOMKINS G. M., *Pleiotypic Response.* « Nature New Biology », *232*, 208-211 (1971).

[48] THOENEN H., ANGELETTI P. U., LEVI-MONTALCINI R. and KETTLER R., *Selective induction by nerve growth factor of tyrosine hydroxylase and dopamine-b-hydroxylase in the rat superior cervical ganglia.* « Proc. Natl. Acad. Sci. U. S. », *68*, 1568-1572 (1971).

[49] LEVI-MONTALCINI R. and BOOKER B., *Destruction of the sympathetic ganglia in mammals by an antiserum to the nerve-growth promoting factor.* « Proc. Natl. Acad. Sci. U. S. », *42*, 384-391 (1960).

[50] ANGELETTI P. U. and LEVI-MONTALCINI R., *Sympathetic nerve cell destruction in newborn mammals by 6-Hydroxydopamine.* « Proc. Natl. Acad. Sci. U. S. », *65*, 114-121 (1970).

[51] ANGELETTI P. U., LEVI-MONTALCINI R. and CARAMIA F., *Structural and Ultrastructural changes in developing sympathetic ganglia induced by Guanethidine.* « Brain Res. », *43*, 515-525 (1972).

[52] BURNSTOCK G., EVANS B., GANNOÑ B. J., HEATH J. W. and JAMES V., *A new method of destroying adrenergic nerves in adult animals, using guanethidine.* « Br. J. Pharmac. », *43*, 295-301 (1971).

[53] THOENEN H. and TRANZER J. P., *Chemical sympathectomy by selective destruction of adrenergic nerve endings with 6-hydroxydopamine.* « Naunyn-Sciemiederberg's Arch. exp. Path. Pharmak. », *261*, 271-288 (1968).

[54] HENDRY I. A., *The response of adrenergic neurons to axotomy and nerve growth factor.* « Brain Res. », *94*, 87-97 (1975).

[55] JOHNSON E. M. and ALOE L., *Suppression of the* in vitro *and* in vivo *cytotoxic effects of Guanethidine in sympathetic neurons by Nerve Growth Factor.* « Brain Res. », *81*, 519-532 (1974).

[56] LEVI-MONTALCINI R., ALOE L., MUGNAINI E., OESCH F. and THOENEN H., *Nerve Growth Factor induced volume increase and enhanced tyrosine hydroxylase synthesis in the chemically axotomized sympathetic ganglia of newborn rats.* « Proc. Natl. Acad. Sci. Wash. », *72*, 595-599 (1975).

[57] ALOE L., MUGNAINI E. and LEVI-MONTALCINI R., *Light and Electron microscopic studies on the excessive growth of sympathetic ganglia in rats injected daily from birth with 6-OHDA and NGF.* « Arch. Ital. Biol. », *113*, 326-353 (1975).

[58] LEVI-MONTALCINI R., *The Nerve Growth Factor: Its role in Growth differentiation and function of the sympathetic adrenergic neuron.* In: « Perspectives in Brain Research », (Corner M. A. and Swaab D. F. eds.), « Progress in Brain Research », *45*, 235-238 (1976).

[59] BIORKLUND A. and STENEVI U., *Nerve Growth Factor: Stimulation of regenerative growth of central noradrenergic neurons.* « Science », *175*, 1251-1253 (1972).

[60] BJERRE B., BJORKLUND A. and STENEVI U., *Inhibition of the regenerative growth of central noradrenergic neurons by intracerebrally administered anti-NGF serum.* « Brain Res. », *74*, 1-18 (1974).

[61] BJORKLUND A., BJERRE B. and STENEVI U., *Has Nerve Growth Factor a role in the regeneration of central and peripheral catecholamine neurons?* In: « Dynamics of Degeneration and Growth in Neurons », (Fuxe K., Olson L. and Zotterman Y. eds.), Proc. Intern. Symp. in Wenner-Gren Center, Stockholm May 1973, « Pergamon Press », 389-409 (1974).

[62] MOMARD D., STOCKEL K., GOODMAN R. and THOENEN H., *Distinction between nerve growth factor and glial factor.* « Nature », *258*, 444-445 (1975).

[63] EBENDAL T. and JACOBSON C.O., *Human glial cells stimulating outgrowth in cultured chick embryo ganglia.* « Zoon », *3*, 169-172 (1975).

[64] HOUWINK R., *The Odd Book of Data.* Introduction Elsevier Publ. C. (1965).