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COMMENTARY

PEPTIDES AND MEMORY

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BIOLOGICAL assays have been traditionally the basic tools of pharmacology, since drugs are defined primarily by their actions on living systems. When, around the turn of the century, the idea of endogenous active substances was gaining ground, the bioassay method was borrowed from pharmacology and became the foundation stone of the doctrine of hormones, antibodies and neurotransmitters, to mention only its most obvious achievements. Biological tests are uniquely suited to demonstrate the presence of minute amounts of biologically active material of unknown chemical composition in complex mixtures such as body fluids or tissues. They are also useful for the detection of substances of known composition for which no suitable physical or chemical methods are available. Last but not least, bioassays are indispensable for guiding the isolation and purification of these substances leading to their chemical identification. Bioassays occupy the central position in the well established research strategy to which we owe the important advances mentioned above. Once the isolation of an active substance is accomplished with the help of the bioassay and its structural identification is achieved, it is usually possible to find a suitable chemical method to replace the biological test. One could say that the final success of a bioassay is to bring about its own obsolescence.

A series of attempts have been made in the last 10 yr to apply the bioassay approach to the biochemical problem of memory. The purpose of this review is to examine some of the aspects of this problem and to discuss the controversy surrounding the use of bioassays for its solution.

THE BIOCHEMICAL PROBLEM OF MEMORY

There are really two biochemical problems of memory: (1) are there any chemical changes in the brain that can be correlated with the acquisition and storage of information? and (2) are these changes information-specific, i.e. do they represent an actual coding of neural information? The first formal hypothesis,[1] inspired by the then emerging idea of a molecular code for genetic information, definitely postulated the information-specificity of the chemical correlates of memory. When Hydén[2] adopted the hypothesis, he assumed that the information reaching the neuron in the form of an electrical pattern would be able to "specify" an RNA sequence and induce the synthesis of the corresponding protein. In his experimental approach to the biochemical problem of memory, he was able to detect quantitative and qualitative RNA changes in neurons isolated from the brain of animals in behavioral training.[3] The work of his laboratory was later extended to proteins but the information-specificity has been gradually de-emphasized and in a recent review of his work he sees

no reason to believe that "the molecule could register a specific memory within its structure".[4] This trend is apparent also among the workers who, following Hydén's experiments,[2] studied RNA and protein metabolism (not in isolated neurons but either in whole brain or selected areas of it) in correlation with learning.[5]

The results of the search for chemical correlates of memory have been supported by experiments using inhibitors of RNA and protein metabolism.[6] Observations of an impairment of learning by these substances suggested that increased RNA and, particularly, protein synthesis were necessary for the consolidation of acquired information but supplied no specific information on the role of the newly synthesized substances.

The logical interpretation of the results obtained by the two approaches just mentioned was that the chemical changes represent only "epiphenomena" of memory rather than "concomitants of the specific information storage processes".[7] At most, they could represent an increased production of "a relatively small number of regulator proteins that might act as receptors for neurotransmitters or as enzymes which regulate the biosynthesis of neurotransmitters or the like".[8] The methodology used could hardly have led to other conclusions. Let us assume, for the sake of argument, pituitary secretion being studied by measurement of RNA and protein metabolism in the gland under a number of conditions such as stress, oestrus cycle, castration, thyroidectomy, metabolic changes, etc. The results would probably indicate increased metabolism but, in the absence of biological tests, could give no hint of the multiplicity of pituitary hormones.

THE BEHAVIORAL BIOASSAY APPROACH

It is probable that chemical coding of a particular information would not represent more than an infinitesimal change in the chemical composition of the brain. Without previous knowledge of the structure of the substances involved, such a change would be below the threshold of sensitivity of the methods used at present. The obvious choice, in a case like this, is an experimental design that includes the following steps: behavioral training of donor animals, extraction of the hypothetical material from the tissues of the trained donors, administration of the extract to naïve recipients and testing of these recipients for behavioral changes related to the training of the donors.

Experiments of this type, first done in planarian worms[9] under the name of "memory transfer", were quickly popularized by the lay press. Neither the authors of the experiments nor their critics, who soon became vocal, seemed to realize the real significance of these experiments and their historical credentials. When, in 1965, the same principle came to be applied to higher organisms, the question still was whether memory can be "transferred" or not (see reviews[10–13]). It was not fully realized that the main significance of this research was to give us an insight into what happens in the brain of learning animals and to lead eventually to the identification of specific substances.

It soon became obvious, however, that these were not simple experiments that could be done by unsupervised beginners with a few leftover animals but required the skilled cooperation of an interdisciplinary team of behavioral scientists, biochemists and pharmacologists. The experimental conditions include critical variables, some of which are still incompletely known: the exact conditions of donor training, preparation of extracts and recipient testing must be determined by trial and error

for each particular assay. Above all, the tests have to be conducted under "blind" conditions and a variety of controls must be devised to specify the significance of the results.[12,13]

Of the 35 laboratories that have published successful bioassays, only six were concerned with the chemical composition of whatever active substance may have been associated with the behavioral change. The others were either not interested or assumed, under Hydén's influence, that the active material was RNA. In general, it was taken for granted that any substances involved in the chemistry of memory must be "macromolecules".[14–16] Nevertheless, our experiments published in 1965 suggested an active substance that was dialyzable and inactivated by trypsin.[17] Further evidence coming from a number of laboratories indicated that the substances formed in the brain of animals learning a new behavior were comparatively small peptides with about 6 to 25 amino acid residues.[18–22]

Up to the present, only one of these peptides has been fully identified. It is the pentadecapeptide named scotophobin, isolated from the brain of rats trained to reverse their natural dark preference and to avoid the dark.[23] It has been reproduced by synthesis and its dark avoidance-inducing action was confirmed in several laboratories.[24–27] Results obtained by a chemical method, developed for its detection and quantitative determination,[22] indicate that the peptide appears in rat brain only during dark avoidance training, reaches a maximum of about 200 ng/g of brain on the sixth day of training, after which it decreases and, at 15 days, cannot be detected any more. Its localization is predominantly cortical, but measurable amounts are present in all parts of the brain. About 1 per cent of the exogenous, intraperitoneally injected scotophobin is found in the brain about 4 hr after injection and by 48 hr, which is the period of its peak behavioral activity, no detectable amounts can be found. It is assumed that the peptide is incorporated into some cellular structures, perhaps synaptic membranes.

Another pure peptide has been isolated from the brain of rats habituated to an acoustic stimulus.[28] It is hexapeptide (pglu-lys-gly-tyr-ser-lys) that has recently been synthesized but has failed to show the full effect of the natural material, which consists in a marked reduction of startle responses to the sound stimulus. Variants of the proposed sequence, including a possible cyclic structure or substitutions on the lysine sidegroup, are now being synthesized and will be tested.

Two peptides are being purified from brains of goldfish trained for avoidance behavior based on color discrimination. One of them has been taken from goldfish trained to avoid the blue compartment of a tank, the other from fish that avoid the green compartment.[29] A third peptide has been isolated from goldfish brain that reproduces a learned adaptation of smimming behavior.[30]

Other training-induced peptides have been demonstrated in the brain of animals trained for step-down avoidance,[12] maze running,[31] red and green avoidance,[19] spinal fixation of postural asymmetry[20] All of them have different molecular sizes and enzyme specificities, suggesting that each type of learned behavior is associated with a distinct peptide sequence.

Control of innate behavior by peptide hormones is, of course, well established and the possibility of their involvement in learned behavior is supported by DeWied's work.[32] He has shown that ACTH and some of its fragments as well as α- and β-melanocyte stimulating hormone and vasopressin have the general effect of consoli-

dating memory and delaying its extinction. This effect is independent of the hormonal action of these peptides and a desglycinamide derivative of lysine vasopressin, isolated from the neurohypophysis, possesses the full behavioral effect. Similar results were obtained by DeWied[32] with some synthetic derivatives of scotophobin.

ANALYSIS OF A CONTROVERSY

It is hard to say what the critics of the behavioral bioassay approach object to most, the method itself or what its results imply, namely the existence of a molecular code of memory. The two have become linked in a sort of vicious circle: the method is objected to because it tends to prove an unpopular hypothesis and the hypothesis becomes objectionable because it is supported by an unpopular method.

The early criticisms were addressed to the method and questioned its reliability (see reviews[12,13]). They were justified when they showed that purified brain RNA preparations did not have the behavioral effects claimed and that, in any case, RNA would not pass the blood–brain barrier. They lost their justification when the active substances proved to be peptides that could be extracted with RNA but were lost if the RNA was too highly purified or the correct conditions of pH were not observed.[33] Yet, these criticisms of the middle sixties had a lasting influence in crystallizing a negative attitude toward the whole approach. Many neurobiologists lost interest in the problem and are unaware of its further developments.

A few more recent replications of behavioral bioassays were unsuccessful because of neglect of some critical experimental conditions.[22,33] It should be stated that, even under the best of conditions, the behavioral bioassays are not 100 per cent successful. In my laboratory, almost 10 per cent of the assays have been negative and the failures could not be explained by any obvious technical fault. Whether the failures are due to seasonal variations in the animals or to some other unknown factor is still uncertain. One should remember that Loewi's famous experiment,[34] which first demonstrated the neurohumoral theory, was considered irreproducible by several reputable physiologists and did not gain complete acceptance until several years later when the "Vagusstoff" was identified with acetylcholine.

It is well known that, even after this explanation, many more years had elapsed before the neurohumoral theory gained full recognition, indicating clearly that the object of the controversy was the new idea rather than the technique that proved it. The situation is probably similar for the molecular code of memory. Other experiments suggesting a role for peptides in memory, like those of DeWied,[32] although based on behavioral bioassays, have not received the same hostile scrutiny because they did not claim any specificity.

The molecular coding hypothesis has been called "fanciful",[33] "implausible", "superfluous"[8] and worse. These opinions stem largely from a fundamental misunderstanding of what the molecular code means. It is represented as conflicting with all the well established notions on the operation of the nervous system and negating the existence of the highly differentiated organization of the brain. The fact is that almost all the molecular hypotheses are based on the existence of specific circuits, which result from the reprogramming of the innate, genetically determined pathways.[35,36] The molecular code would merely mark the new connections that are created by learning and assure their preservation as memory.[37–39].

It has recently been said that the experiments we are discussing represent "premature discoveries" which the scientific community is not prepared to accept because they are incompatible with "canonical knowledge".[40] This is true only because some scientists have misinterpreted the bearing of molecular coding on our understanding of neural function, in the same way in which the meaning of the neurohumoral theory was misinterpreted in the twenties or thirties by some Sherringtonian fundamentalists.

PERSPECTIVES

Work on the molecular code of memory appears to many either as a blind alley or a radical revolution. It is really neither of these; it represents merely a further penetration of chemical principles into our understanding of neural function. It has taken over half a century to change the image of the nervous system as a purely physical, electrical machine into an information-processing device that is fueled by chemical energy, whose "switches" are chemical, and in which impulses are propagated not by the rapid streams of electrons but by the much slower transport of ions. The hypothesis under discussion would go one step further by postulating that the device is programmed genetically by chemical labeling of the innate pathways and is being continuously reprogrammed by a molecular code derived from this labeling system.[39]

Admittedly, the hypothesis is still in its infancy. The principle of "one behavior, one peptide" that could be regarded as its basic postulate remains to be convincingly demonstrated. The idea, however, is in the mainstream of neurobiological thinking, in spite of some opposing opinions. The "chemicalization" of the nervous system is of particular importance to pharmacologists. One could make the general statement that drugs can act only by interfering with biochemical processes. Every time a new chemical process is discovered in living systems, pharmacology gains a new target for drug action. At present, the neurohumoral principle can account for the action of drugs on the affective components of behavior. The possibility of a molecular code of memory opens up the perspective of a psychopharmacology of the cognitive functions.

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