This Week's Citation Classic


The development and application of a method for the specific localization of vasopressin and oxytocin in the rat brain by immunofluorescence is described. The topographic distribution of these neuropeptides is given not only in the classical neuroendocrine cells of the supraoptic and paraventricular nucleus, but vasopressin was also found in the suprachiasmatic nucleus. [The SCI® indicates that this paper has been cited in over 175 publications.]

Immunofluorescence of Vasopressin and Oxytocin

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This paper and reference 1 deal with the development and the application of a procedure for the specific immunocytochemical localization of vasopressin and oxytocin in the rat brain, at the Netherlands Institute for Brain Research. I wrote the first draft of this paper during a short Christmas holiday in Paris in 1974, exactly two years after J.L. Touber (University Clinic for Internal Medicine, Amsterdam) suspected his animal keeper had killed a few of his rabbits that were immunized against vasopressin for an economical Christmas dinner. Investigation of their hypothalamus, blood, and urine revealed, however, that these animals suffered from a severe diabetes insipidus due to excellent antibodies against vasopressin.

In August 1973 I went to T.E.W. Feltkamp of the Central Laboratory of the Red Cross with far too many of these antibodies in a Dewar vessel and followed the procedure they routinely used to determine autoantibodies in serum of patients. The next day I found for the first time (by immunofluorescence) vasopressin in cells of the supraoptic nucleus (SON).

Following improvements of the fixation procedure, I wrote a thesis proposal on this line of work, but our director at that time, J. Ariëns Kappers, decided to give priority to his own interest in the pineal gland. In 1974, as a consolation, I was allowed to employ C.W. Pool as a part-time student for the development of immunocytochemistry. He has contributed enormously to the immunocytochemical research in our institute. In spite of other because of Ariëns Kappers' decision, we dedicated our paper to him on his 65th birthday. Later he told me he had enjoyed this present very much.

Touber's group showed by radioimmunoassay that our antibodies were specific for vasopressin. Yet, we got strong staining in the hypothalamic of a homozygous diabetes insipidus rat. This "Brattleboro" mutant is not capable of producing vasopressin. Using peptides on agarose beads (a model system that P.J.A. Capel presented during the Fifth International Conference on Immunofluorescence and Related Staining Techniques), it was shown that this staining was due to cross-reactivity with the related peptide oxytocin, and a solid phase procedure for antibody purification was developed.

Our findings on the topographic distribution of vasopressin and oxytocin in the SON and paraventricular nucleus (PVN) showed that each cell contained one hormone (i.e., vasopressin or oxytocin) and that vasopressin cells were localized more caudally and oxytocin cells more rostrally; and our findings contradicted the "classical" view that the SON would predominantly or entirely synthesize vasopressin and the PVN oxytocin. These results are mentioned in 66 percent of the citations to this paper. In addition, vasopressin was found in neurons that were not neurosecretory in nature, i.e., in the suprachiasmatic nucleus. This new and important aspect is only mentioned in 10 percent of the citations.

Yet, our paper thus became the start of a number of well-cited papers from our group on extrahypothalamic sites of production of these neuropeptides, their transport by nerve fibers to other brain areas, and release by synaptic contacts currently also in the human brain.

However, the important methodological point of our papers, that data on the potency or specificity of an antibody in a radioimmunoassay do not give any information on its immunocytochemical properties, and the problem of cross-reactivity of related peptides and the necessity to use solid-phase adsorption for purification of antibodies against peptides, did not get across sufficiently. Papers overlooking these problems are still published regularly. It is remarkable that these aspects have low citation scores (in 2 percent and 4 percent of the citing papers, respectively). It is hard to believe that this is because the message was not brought out clearly in our paper, especially since our group has since then repeated this message over and over again in courses, reviews, and other papers with only limited practical success. It is probably just more convenient and attractive to apply an antibody and describe the results instead of carrying out painstaking work to find what is the substance one has actually stained using a combination of separation techniques and immunocytochemistry.


This Week's Citation Classic


HLA typing of 150 patients with early onset diabetes mellitus showed a significant association with B8 and Bw15. Determination of the HL-A haplotypes inherited by siblings affected with this type of diabetes showed an increase in identity of haplotypes above random expectation, strongly supporting the existence of HL-A linked disease susceptibility genes. [The SCI® indicates that this paper has been cited in over 205 publications.]

New Paths in Diabetes Genetics

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For several years Cyril Clarke and his associates in the Department of Medicine at the University of Liverpool had been interested in the possible role of genetic polymorphisms in the pathogenesis of disease, and studies were carried out involving the ABO and other blood group systems.

When the HL-A polymorphism was revealed, it seemed to many people likely that association studies of various clinical disorders might well reveal the existence of disease susceptibility genes. The striking association of B27 with ankylosing spondylitis had just been reported. With the help of good friends at the Blood Transfusion Centre in Bristol who supplied typing sera, we were soon able to tissue type. I was at the time developing a rheumatology service, and my first research reports concerned HL-A in Reiter's syndrome and anterior uveitis and one of the early studies of HL-A in psoriasis.

Andrew G. Cudworth was working on diabetes mellitus in the department, and discussions with him regarding the genetics of diabetes led us to think that an HL-A association study of the two main clinical types of diabetes might be fruitful. The finding of an apparent association of B8 and Bw15 in the first extended study.

Linkage and association for discussion in the deep were we also being doing analyses in families with insulin-dependent Penrose had proposed a s of testing for linkage. (1) demonstration of auto bio in the Luthera and used this approach.) The of Type I diabetes tenc haplotypes gave strong HL-A linked susceptibility method of analysis, put f Green, gave further pub HLA and disease studies.

Cudworth subsequent mew's Hospital, where h an extensive study of ti islet-cell antibodies in fa his untimely death reap area of clinical research. Following these early m methods have been appl disorders. It was soon af of inheritance of HL-A ha would throw considerable haviour (dominant, recess susceptibility genes. (2) Good number of unkn gene frequency, recombin heterogeneity, a form anal has been nee and several other gen important contributions.

The other major area of application of increasing histocompatibility complex cellular typing methods length polymorphism us allowed for increasingly haplotypes that increase to Type I diabetes. It is to become very complex and John A. Todd's rec situation suggests that i will be necessary before genetic structure of Typ

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