PHYSIOLOGICAL PEPTIDES AND NEW TRENDS IN RADIOIMMUNOLOGY

Ch. A. Bizollon Editor

PHYSIOLOGICAL PEPTIDES AND NEW TRENDS IN RADIOIMMUNOLOGY

Proceedings of the Vth International Symposium on Radioimmunology held in Lyon (France) 9-11 April 1981.

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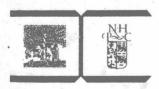
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PREFACE

ACKNOWLEDGEMENTS

This volume serves as the proceedings for the Vth International symposium on Radioimmunology of Lyon (France) which successfully took place on April 9-11 1981. These symposia, which started in 1972 soon after the wide development of radioimmunoassays in France, were firstly devoted to the technical problems of the methodology. In the following years it appeared to us useful to join together the methodological and pathophysiological aspects of the research made available by radioimmunology.

One of the major interests of these meetings was not only the possibility of lity of direct and friendly contacts, but above all the possibility of comparison between the procedures used and the data obtained in different laboratories. This was the aim of a satellite Session (held in the "Centre de Médecine Nucléaire" Lyon) devoted to the critical parameters of a TSH radioimmunoassay as they could be defined by a collaborative study developed by more than 90 European laboratories.

The conclusion of this Session could be that the adolescent (i.e. ra-MINIGO dioimmunoassay) suffering from gigantism described a few years ago by MARDA 130 G.E. Abraham has now the size and the experiences of an adult.

During the symposium in which 450 scientists of 21 countries partici- MOZIID pated the presented papers dealt with the role of brain peptides, growth GXAJD factors, free form of hormones and drugs, and monoclonal antibodies, this last topic being now at the frontier of the radioimmunology.

It is a great pleasure for the Organizing Committee to acknowledge its great debt to Pr M. Jouvet for his excellent opening lecture on "Neuropeptides and Sleep", to Pr P. Franchimont, Dr P. Chatelain, Pr R.P. Ekins and Pr J.P. Revillard for chairing with attention and skill the sessions of the Symposium.

Our thanks go also to those whose moral and financial support allowed the broad international representation gathered in this meeting, especially the Hospices Civils de Lyon and La Chambre de Commerce et d'Industrie de Lyon. We extend our gratitude to Dr J. Geelen from Elsevier/Nord-Holland for his presence during the Symposium and the careful attention to the production of this volume of our symposium.

ACKNOWLEDGEMENTS

PREFACE

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The Organizing Committee

Michel JOUVET, Department of Experimental Medicine, INSERM U 52, CNRS LA 162, Claude-Bernard University, LYON, France.

Summary

The difficulties and pitfalls of the research concerning the relationship between neuropeptides and sleep are outlined. First, since sleep is not an homogenous state but is composed of two different states which can be separately altered, there may be different peptides acting upon Slow Wave Sleep (SWS) or Paradoxical Sleep (PS) mechanisms. If SWS is enhanced selectively, there is some risk that slow cortical activity could be provoked by unphysiological mechanisms since many drugs can increase slow cortical activity without being really hypnogenic. Secondly, sleep being a spontaneous phenomenon which occurs usually during more than 12 hrs a day, it is not the induction of sleep which is important but merely the increase of both SWS and/or PS. Since most laboratory animals (rats, mice) sleep less during nights, it will be easier to disclose hypnogenic effect by injecting a peptide before night time. If hypnogenic peptides exist they should fulfill the following criteria: they should be isolated and characterized in the brain and/or the CSF and/or the blood; they should have a dose dependant effect; they should increase either SWS, PS or both; they should be active in different mammalian species and finally their inactivation should induce insomnia.

At the present time it is likely that some peptides modulating the sleep waking cycle have been isolated but is is not yet proven that they play a necessary role in sleep: the following results are briefly summarized.

I - Are hypnogenic peptides really hypnogenic ?

Delta Sleep Inducing Peptide (DSIP) was the first peptide related with sleep mechanisms isolated by MONNIER and his group. It has some enhancing effect upon SWS or PS in rabbits, mice and cats. Negative results have been reported in rats.

Arginin vasotocine which could be released from the subcommissural organ (according to PAVEL) has some SWS-inducing and PS-suppressing effects in the cat. It has no significant effect in the rat.

II - Hypnogenic transferable factors : are they peptides ? TAROUNIAN BRAHT BRA

Transfer of CSF or Brain Stem extracts from sleep deprived goats or rats in the ventricular system (or intraparenterally) or recipient rats or mice may increase either SWS or SWS and PS. This suggests that some factor might indeed accumulate in the brain during prolonged wakefulness. Such a concept is reminiscent of PIERON's "hypnotoxines". Although the molecular weight of both CSF and brain stem extracts are similar (300-500 daltons) these factors have not yet been characterized.

The mechanisms of action of putative sleep peptides are discussed in the light of the monoaminergic theory of sleep. It is possible that serotonin might act first by initiating the synthesis or the liberation of some factor which in turn would initiate the onset of SWS and PS. Indeed the intraventricular injection of CSF from PS deprived cat is able to restore PS in recipient cat made totally insomniac by a previous injection of P.chlorophenylalanin which inhibits the biosynthesis of serotonin.

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BRAIN PEPTIDES

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RADIOIMMUNOASSAY OF SOMATOSTATIN: METHODOLOGICAL PROBLEMS AND PHYSIOLOGICAL INVESTIGATIONS

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INTRODUCTION

Somatostatin was originally isolated from sheep hypothalami on the basis of its growth hormone (GH) release inhibiting activity and characterised as a tetradecapeptide maintained in a cyclic conformation by an intramolecular disulphide bridge between two cysteine residues 1.

H₂N-Ala-Gly-Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys-COOH

The peptide was subsequently shown to have a broad range of inhibitory actions on pituitary, pancreatic and gastrointestinal hormone secretion, and on gastrointestinal exocrine functions 2,3 . It has direct effects on the central nervous system (CNS) affecting both behaviour and neuronal activity and in smooth muscle it inhibits neuronal release of adrenergic and cholinergic transmitters 5 .

Somatostatin has a wide distribution in the CNS and gastro-intestinal tract. In the CNS it occurs in both hypothalamus and extrahypothalamic brain, where it has been localised to the synaptosome fraction, and in spinal $\operatorname{cord}^{6-9}$. In the gastro-intestinal tract somatostatin is present in substantial amounts in the pancreas, stomach and intestines, where it has been localised within the endocrine-like D cells^{7,8,10-12}. Somatostatin has also been demonstrated in peripheral nerve fibres in the intestinal wall¹³.

The widespread distribution of somatostatin together with its broad spectrum of biological actions has led to the suggestion that it has several physiological roles. Thus, hypothalamic somatostatin may be an important modulator of pituitary GH and TSH release, while somatostatin in the extrahypothalamic brain and

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peripheral nerves may play a neurotransmitter and/or neuro-modulator role. Somatostatin in the D cells can be released into either the interstitial space, or into the circulation and it has been suggested that pancreatic and gastrointestinal somatostatin may regulate the release of insulin, glucagon and gastrointestinal hormones by paracrine and/or endocrine mechanisms.

In order to investigate the possible physiological roles of to somatostatin in man, we have developed a radioimmunoassay (RIA) for somatostatin in human plasma, and used it to investigate possible factors influencing somatostatin secretion.

RADIOIMMUNOASSAY OF SOMATOSTATIN

Antiserum to somatostatin was raised in rabbits and showed no cross-reaction with a wide range of hypothalamic, pituitary or gastrointestinal hormones. The antiserum is directed towards the central part of the molecule containing the tryptophan residue, and shows approximately 60% cross-reaction with dihydrosomatostatin. Tyr 11 somatostatin was iodinated using lactoperoxidase and purified on a column of octadecasilyl (ODS) silica. This method was found to be superior to purification of the iodination products on CMC cellulose and gave a stable tracer with a storage time of up to three months at $-70\,^{\circ}\mathrm{C}^{14}$.

RIAs for somatostatin in tissue extracts and in perfusion fluids from in vitro assay systems have been developed in several laboratories and are relatively easy to perform, however the measurement of somatostatin in plasma has proved difficult for a number of reasons. 1) Iodinated somatostatin is rapidly degraded in the presence of plasma or serum 15,16. 2) There is evidence that somatostatin binds to high molecular weight (MW) proteins in plasma and serum 17. 3) Somatostatin circulates in low concentrations in peripheral blood.

Despite these difficulties, a number of RIAs have been developed for somatostatin in unextracted plasma or serum. In these assays, degradation of iodinated somatostatin has been reported to be overcome by using EDTA in the assay buffer, and Trasylol in the blood samples 15,18 .

To investigate the effect of plasma on binding of somatostatin tracer to antiserum, we prepared somatostatin-free plasma using

immunoaffinity chromatography 14, and constructed a series of standard curves in buffer, and in somatostatin-free plasma with and without the addition of Trasylol and EDTA. As shown in Figure 1, somatostatin-free plasma caused a marked reduction in tracer-antibody binding and this could not be completely overcome by the use of Trasylol and EDTA. Similar results have also been reported in rat plasma 16. The discrepancy remaining between standard curves in buffer and in somatostatin-free plasma in the presence of both Trasylol and EDTA could be due to incomplete prevention of tracer degradation, or to the presence of high MW somatostatin binding proteins in the plasma which compete with the antibody for tracer binding. In agreement with others 17 plasma samples measured using an unextracted assay system did not show parallelism with standard somatostatin, furthermore, estimated fasting levels of 80 - 150 pg/ml in normal subjects were significantly higher than those subsequently obtained for the same samples using an extracted assay system.

In order to overcome these problems, we elected to extract somatostatin from plasma prior to RIA. Vycor glass, a leached silica glass which has been successfully used for the RIA of a number of peptide hormones was chosen for use, and the method has been described in detail 14. Using this technique the problems of tracer degradation, and possible interference by somatostatin binding proteins are overcome, and all plasma samples diluted in parallel with a cyclic somatostatin standard curve. By using an initial volume of 2 - 4 ml of plasma a large concentration factor is achieved, thus effectively increasing the sensitivity of the assay and permitting detection of small changes in plasma immunoreactive somatostatin (IRS) levels. Using this assay, fasting plasma IRS levels were measured in 40 normal subjects. The mean level was 34 pg/ml, with a range of 17 - 81 pg/ml.

RESPONSE OF CIRCULATING IRS TO HORMONAL STIMULI IN MAN

As discussed in the introduction, somatostatin has been suggested to play a physiological role in the control of insulin, glucagon and GH secretion. However whether endocrine control mechanisms, or local control mechanisms are involved is not clear. If a general endocrine action of somatostatin is important,

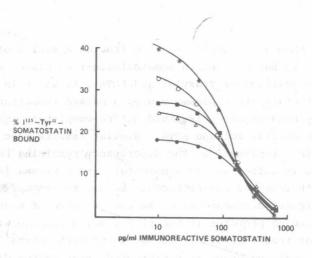


Fig. 1. Standard curves of cyclic somatostatin diluted in buffer or plasma with or without the addition of trasylol or EDTA.

Standard diluted in:	Diluent
- 0.05 M phosphate buffer pH 7.4 containing 0.025 M EDTA + 2000 KIU/ml trasylol	0.05 M phosphate buffer
• - • Somatostatin-free plasma	0.05 M phosphate buffer
Δ - Δ Somatostatin-free plasma	0.05 M phosphate buffer containing 0.025 M EDTA
Somatostatin-free plasma containing 2000 KIU/ml trasylol	0.05 M phosphate buffer
o - o Somatostatin-free plasma containing 2000 KIU/ml trasylol	0.05 M phosphate buffer containing 0.025 M EDTA
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changes in circulating levels of insulin, glucagon and GH might be expected to produce changes in circulating somatostatin levels by a feedback mechanism. In order to investigate this, the response of IRS to hormonal stimuli was studied in normal human volunteers.

Insulin-induced hypoglycaemia

Fasting volunteers received intravenously, on separate occasions, either insulin (0.15 U/kg) or normal saline. As shown in Figure 2, after the insulin injection plasma IRS levels showed a marked and sustained rise, and were significantly higher than control values between 45 and 150 minutes after the injection

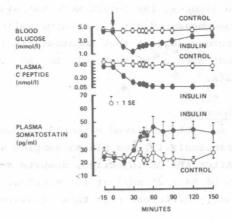


Fig. 2. Changes in blood glucose and plasma C-peptide and IRS after intravenous insulin (\bullet - \bullet , n = 10) or normal saline (o - o, n = 6) given at 0 min (\bullet). Vertical bars are 1 standard error of the mean.

(p < 0.05). The reason for the rise in plasma IRS seen following intravenous insulin is not clear at present, however it does not appear to be merely a stress phenomenon as no rise in plasma IRS was seen in patients undergoing surgical stress despite a large rise in plasma cortisol levels 19 . Neither does the rise in IRS appear to be due to the fall in blood glucose, as a rise in plasma IRS was also seen after oral, but not intravenous, glucose 20 . Insulin induced hypoglycaemia produces a rise in gastric acid secretion in normal subjects, and Skare and coworkers have demonstrated that stimulation of gastric acid secretion produces a rise in IRS levels in human peripheral plasma and gastric juice 21 . This suggests that it may be the stimulation of acid secretion by insulin hypoglycaemia which was responsible for the observed rise in plasma IRS.

Exogenous glucagon

Plasma IRS levels were measured in fasting subjects following a subcutaneous injection of 1 mg glucagon. Despite a typical blood glucose response (mean \pm 1 SE, 7.6 \pm 0.7 mmol/l at 45 minutes and 3.4 \pm 0.1 mmol/l at 210 minutes) and a rise in GH to