Medical Research Archives

European Society of Medicine

OPEN ACCESS

Published: November 30, 2023

Citation: Rehfeld, J.F. et al. 2023. The Threat of Commercial Hormone Kits to Endocrinology. Medical Research Archives, [online] 11(11). https://doi.org/10.18103/mra.v11i11.4592

DOI: https://doi.org/10.18103/mra.v11i11.4592

ISSN: 2375-1924

EDITORIAL ARTICLE

The Threat of Commercial Hormone Kits to Endocrinology

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ABSTRACT

Over the last decades, the market for commercial hormone kits has grown considerably. The kits are today used extensively in endocrine research and diagnostics. Unfortunately, a significant fraction of the kits are unreliable in both analytical and diagnostic terms. Consequently, the aim of the present article is to draw critical attention among endocrinologists to the reliability of commercial hormone kits. For that purpose, we have briefly reviewed the kit situation for four different hormones: The brain-gut hormone cholecystokinin (CCK); the antral hormone gastrin; the pancreatic hormone glucagon; and the cardiac natriuretic hormones. We conclude that the situation for the described peptide hormones probably shows only the tip of an iceberg. Therefore, we strongly recommend kit producers examine their products carefully with respect to the new endocrine biology and diagnostic reliability before the kits are marketed. Moreover, we also recommend quality regulation of the kit market in line with the regulations applied to the pharmaceutical drug market.

Introduction

Hormones are per definition blood-borne messenger molecules. Measurement of hormone concentrations in plasma is consequently a fundamental cornerstone in basic and clinical endocrinology. The measurements of the low plasma concentrations of peptide hormones (low picomolar levels) became possible with the invention of the radioimmunoassay (RIA) technology in the early 1960s.[1] Subsequently, further development of immunoassay technologies for instance the enzyme-linked immunosorbent assay (ELISA) also became attractive.[2]

From the beginning, the hormone assays were established as in-house immunoassays in laboratories at university institutes and university hospitals. Many of these laboratories were also involved in basic and/or clinical endocrine research that recognized and often contributed to the understanding of the cellular and molecular complexity of hormone systems as we know them today.[3] The complexity comprises the multistep cellular processing of prohormones leading to biosynthesis of often several bioactive forms of a hormone (molecular heterogeneity); the structural homology between different hormones (hormone families); the widespread expression of the same hormone gene to different cell types in the body; and the cell-specific prohormone maturation and secretion in normal and diseased cells. On top of the molecular complexity of a hormone per se, it also requires recognition that a given peptide hormone often is an agonist for several receptors. Somatostatin, for instance, has five receptors, and cholecystokinin (CCK) two.

Alongside recognition of the fundamental biological complexity of hormone systems, the academic laboratories designed and developed their in-house immunoassays to have the necessary analytical specificity and sensitivity for accurate plasma measurements. In addition, they also ensured that the diagnostic specificity and sensitivity of the measurements were useful in clinical endocrinology and other clinical specialties dealing with hormones such as for instance gastroenterology.[4,5]

The story about deeper insight and improved reliability of hormone measurement, however, began crumbling three to four decades ago. The culprits were, and still are, commercial immunoassay kits that have been insufficiently validated with respect both to analytical and diagnostic accuracy. It has simply become too easy just to raise antibodies of poorly defined epitope specificity without taking neither the basic molecular complexity of hormones nor the alterations during disease into account. Also, several laboratories in science institutes and in hospitals have been naïve and without critical insight into the requirements of quality and reliability of the growing number of commercial kits. The effect of ignorance has in many instances been disastrous.[6] In the following, we describe the problems encountered for plasma measurements of four different peptide hormone systems: cholecystokinin (CCK); gastrin; glucagon; and natriuretic hormones.

1. CHOLECYSTOKININ

Intestinal CCK is secreted from endocrine I-cells. Moreover, plasma CCK originates almost entirely from these cells. CCK regulates gallbladder contraction and pancreatic enzyme secretion. It is also a major satiety signal from the gut to the brain, and besides a widespread transmitter in the central and peripheral nervous systems. Accurate measurement of CCK in plasma has so far been a major challenge for gastrointestinal endocrinology and for endocrinology in general. There have been several attempts to develop plasma CCK assays since the structure of CCK was identified.[3] But the problem has remained and today it is 2-fold: On one hand, many earlier reports on plasma CCK are incorrect because the assays did not possess the necessary specificity and sensitivity. For instance, only few plasma CCK assays have examined the cross-reactivity with sulfated gastrins, which may pose a considerable problem. Consequently, incorrect statements about CCK as a hormone are still published in textbooks and articles. On the other hand, there is a need for accurate assays to settle the role of circulating CCK.

Many laboratories interested in CCK have tried to establish radioimmunoassays (RIAs). The first difficulty in this endeavor was the shortage of peptides for immunizations. Only limited amounts of pure natural CCK have been available. CCK-8 was synthesized in the early 1970s, but it has not been possible to raise antibodies against CCK-8 that did not also cross-react with gastrin. There is simply too little CCK-specific structure in the sequence of CCK-8. Another difficulty has been the isotope labeling

because methionine residues are so easily sulfoxidized. CCK-8 contains 2 methionyl residues and CCK-33 contains 3. That problem has been solved by monooxidative Bolton-Hunter labeling. The largest difficulty, however, is that the antibodies should bind the "active site" of CCK without, as mentioned, binding the homologous gastrin.

Among published studies from the last two decades, 17 studies had used two accurate "in-house" RIAs or a highly sensitive but laborious bioassay. These three "in-house" assays agree closely regarding CCK secretion and plasma concentrations in man. Hence, they measured similar low basal concentrations and food-stimulated CCK responses. Their message is that the CCK concentrations both in the fasting state and after intake of mixed meals are the same in lean and most obese people. Elevated concentrations are seen only in morbid obesity. On the other hand, kit-based studies suffer from two major problems. The first is that some of the kits measure up to 30,000-fold higher concentrations than those described in validated "in-house" assays. Moreover, several kits are marketed with so insufficient specificity documentation that the reasons for the mismeasurements are difficult to establish.[7]

The other problem is a business attitude which copes poorly with both medical research and diagnostics. Hence, some companies have suddenly discontinued the production of CCK kits without warning to either customers/users or producers of the antisera. Such discontinuation was recently experienced with EuroDiagnostica in Malmö, Sweden (now renamed SVAR Life Science AB) and Alpco Diagnostics in Windham, NY, USA. The paradox is that these two companies produced fairly reliable CCK kits and that they had full access to further supplies of a specific antiserum in their kits. The result of their lightning discontinuation is that a number of projects at universities and hospitals have been endangered and some even terminated prematurely. Such behavior calls for a regulation of the market for diagnostic kits in a manner similar to that of the pharmaceutical drug market.[7]

2. GASTRIN

Gastrin regulates gastric acid secretion and gastric mucosal growth. By far most gastrin is synthetized and released from endocrine G-cells in the antral and duodenal mucosa. Gastrin is molecularly highly heterogenous. It circulates in eight different forms (gastrin-71, -34, -17, and -14, each of these occurring in O-sulfated and non-sulfated variants). It is possible to raise antibodies against the common C-terminal epitope in a way where all the variant forms are measured.[8,9] The immunoreactivity of the O-sulfated variants, however, have rarely been examined, although sulfation influences the immunoreactivity significantly.[10]

At present, most of the basic biochemistry and physiology of gastrin have been elucidated.[9] Nevertheless, accurate measurements of plasma-gastrin are still required for proper diagnosis and therapy-control of endocrine tumors that produce gastrin, i.e., gastrinomas. The cause of far most Zollinger-Ellison syndrome in patients is gastrinomas. Four decades ago, commercial gastrin kits entered the market in RIA or ELISA versions. Subsequently, endocrinologists and gastroenterologists world-wide observed patients with classic Zollinger-Ellison symptoms that were reported to have but normal or low concentrations of gastrin in plasma or serum. Undiagnosed gastrinomas have serious and even fatal consequences. Therefore, comparison of commercial gastrin kits with a fully examined in-house gastrin-RIA was initiated. It showed that seven of twelve commercial kits were unreliable. They measured either "false" low or "false" high gastrin concentrations.[5] This result was followed up in several gastrointestinal journal editorials. But there are still unreliable gastrin kits on the market.

3. GLUCAGON

Glucagon is secreted from pancreatic α-cells. Due to its metabolic actions in the liver and for weight reduction, interest in its biology and pathophysiology is increasing. In the α-cells, glucagon is processed from proglucagon to glucagon, and this is dependent on prohormone convertase 2. Measurement of glucagon is challenging due to the amino acid similarities between other proglucagon-derived hormones such as oxyntomodulin and glicentin,[11] and as plasma concentrations reach very low levels (<1pmol/L) during hyperglycemia in healthy individuals. Numerous reports have found that a shift in few amino acids of the epitope of the antibody may cause false high concentrations.[12] This can be exemplified by the introduction of suddenly 5-10-fold higher plasma concentrations

using a commercial RIA (the most frequently used in the literature) due to implementation without careful validation of a new antibody.[13] The existence of so-called gut-glucagon has been of great debate due to these inherent challenges when measuring glucagon. Another recent example on the pursuit of accurate measurement of glucagon is the observation that weight and glucose-lowering drugs may increase glucagon.[14] However, this was later shown to be due to cross-reactivity to other peptide hormones.[15] A final example is that some glucagon kits are unable to measure the physiological dynamics that occur during hyper- and hypoglycemia.[16] In other words, choosing the wrong kit may wrongfully eliminate the basic concept of glucagon in human biology.

4. NATRIURETIC PEPTIDES

Measurement of natriuretic peptides, e.g., atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP), has been one of the recent successes in hormonal measurement for diagnostic purposes.[17] In systolic heart failure, the cardiac myocytes increase their expression and release of both ANP, BNP and their precursors. Conversely, low concentrations of the peptides in plasma basically exclude a diagnosis of heart failure. As heart failure remains a syndrome with diffuse symptoms, the identification of natriuretic peptides in plasma was a game changer in terms of screening and testing patients at risk of heart failure.

In the early phases, measurement of natriuretic peptides in plasma was, in principle, a discipline for a few specialized laboratories at university hospitals. Notably, issues with emerging commercial methods were even then reported.[18] With the huge clinical potential, automated methods were also developed. As with the other hormones mentioned above, the reliability and specificity of the methods used then became a growing concern.[19] First and foremost, the advent of commercial methods challenged the nomenclature for what is actually measured by the methods. From the BNP gene expression, two major peptides were identified in plasma; The C-terminal bioactive BNP peptide hormone, and an N-terminal fragment often named N-terminal proBNP – or just NT-proBNP or N-BNP.[19] However, methods for both peptides also co-measure the intact precursor peptide (proBNP), which has been shown to be the dominant form in plasma from patients with heart failure.[20,21] Thus, commercial kits calibrated with synthetic fragments were and still are, per definition, biased and do not report the true concentrations of peptides in plasma. Moreover, the use of incorrect nomenclature has caused considerable confusion among clinicians, which may even have caused incorrect decision-making by clinicians.

A different issue concerns the post-translational processing of the prohormone structures in the cardiac myocytes. While the commercial kits were developed to measure peptides without modifications, we and others have shown that natriuretic peptides and their biosynthetic precursors are extensively glycosylated.[22,23] These modifications most often involve the epitopes for which commercial antibodies are raised. Thus, methods today still report on non-glycosylated peptides in circulation and simply miss the modified forms. While this does not change the current clinical performance of the current methods, it does open up for methodological improvement. In particular, peptide modification is nearly always affected by disease-causing altered gene expression, and this common biological feature could be used for better diagnostic performance.[24] Appreciating peptide modifications may lead to methods that will better reflect disease states where the current methodology is hampered by what is referred to as “gray zones”, e.g., the current performance of immunoassays is not optimal. Commercial kits for natriuretic peptide measurement have, however, not addressed this challenge and thus still rely on academic laboratories to develop and produce immunoassays against complex peptide hormone systems.

5. CONCLUSION

The nature and extent of the damage of inaccurate commercial hormone kits has been described here for just four hormone systems with which we happen to have long-term experience. They are probably only the tip of the iceberg that threatens endocrinology. In order to meet this threat, we strongly recommend that producers of hormone kits carefully study the biology and clinical significance of the particular hormone systems for which they want to produce kits. Moreover, we also recommend quality regulation of the commercial market for diagnostic kits in parallel with regulation of the pharmaceutical drug market.

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