ENDOCRINE TOXICOLOGY

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CAMBRIDGE
UNIVERSITY PRESS

Published by the Press Syndicate of the University of Cambridge
The Pitt Building, Trumpington Street, Cambridge CB2 1RP
40 West 20th Street, New York, NY 10011–4211, USA
10 Stamford Road, Oakleigh, Victoria 3166, Australia

First published 1992

Printed in Great Britain at the University Press, Cambridge

A catalogue record for this book is available from the British Library

Library of Congress cataloguing in publication data

Endocrine toxicology/edited by Christopher K. Atterwill and John D. Flack.
p. cm.
ISBN 0 521 40225 5 (hardback)
1. Endocrine toxicology. I. Atterwill, C. K. II. Flack, John D.
[DNLM: 1. Endocrine Glands—drug effects. 2. Endocrine Glands—physiopathology. 3. Toxicology. WK 100 E5273]
RC649.E524 1992
616.4—dc20 91—23113 CIP

ISBN 0 521 40225 5 hardback

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Introduction

Two inter-related questions arise from the contents of the various chapters in this book. First, what place does endocrinology have in toxicology, and how can endocrinological effects be characterized and their importance assessed during the course of toxicological studies? Secondly, what is the importance of toxicology in endocrinology and what can toxic effects tell us about the control and responses of endocrine tissues and their targets under both normal and abnormal circumstances? The answer to these questions, which have importance not only for clinicians and toxicologists but also for basic researchers in the field of physiology, will eventually need to be expressed in terms of receptocytes, receptoactivation, signal transduction, mechanisms of hormone secretion, molecular biology, etc.

The endocrine system is heavily involved in homeostasis. In this context the distinction between endocrinology and the rapidly expanding knowledge of regulatory peptides is quite unclear. The term 'endocrine pathology' is seemingly limited to changes in the function and structure of classical endocrine glands and their more obvious targets. However, if its meaning were extended to embrace the effects of disturbance of regulatory peptide function, then it is likely to transpire that endocrine disturbance in this broader sense would be seen as being very widely implicated in toxicology.

There are four steps in the investigation of potential endocrine toxicity. First, appropriately sensitive clinical and pathological techniques for the detection of such toxicity need to be available and applied. Secondly, the evaluation, nature and magnitude of effects need to be measured. Thirdly, mechanisms need to be understood. And finally, the likely significance of an effect in terms of general health and wellbeing and of possible late effects on target organs needs to be assessed.

Detection of endocrinological toxicity

As exemplified in almost every chapter in this book, effects of exposure to drugs, toxins, or other agents may be functional and/or structural in nature. Both functional and structural changes may lie within the range of normal physiological action and reaction (e.g. effects on oestrous cycling or a change in the circulating level of T_4 due to a rise in thyroxin binding globulin following exposure to an oestrogen). Alternatively, effects may be frankly abnormal (e.g. the sharp rise in circulating prolactin produced by a dopamine antagonist, or the reduction in circulating cortisol [or cortisone, depending on the species] caused by a dehydrogenase inhibitor). If the action of the toxicant is sufficiently prolonged and/or severe, then persistent morphological abnormalities are likely to develop in one or more organs of the endocrine orchestra or their target tissues. In these circumstances the histopathologist will report pathological change.

Effects on the endocrine system may be direct (e.g. the agent may have oestrogenic activity) or secondary to an effect on a target tissue for a hormone (e.g. destruction of the seminiferous tubules in the rat testis leads to the appearance of castration cells in the pituitary gland).

The inadequacy of routine toxicological procedures with regard to the evaluation of effects on endocrine status

The usual first main purpose of toxicity tests is to detect and quantify adverse effects on behaviour, clinical appearances, bodily functions and the macroscopic and microscopic appearance of tissues. In many cases there is no *a priori* reason to expect any particular adverse effect, so that when an adverse effect is found at necropsy it may come as a surprise or, should we say, shock. Observations made during the in-life phase of studies may alert one to the probability of finding pathological changes in particular tissues. However, it is not at present usual practice routinely to measure the levels of circulating hormones. Consequently most toxicological effects on endocrine function and status are discovered for the first time at necropsy. There are, of course, notable exceptions to this. For instance, a high incidence of mammary tumours in female rats will lead one to expect to find a high incidence of prolactinomas of the pituitary gland at necropsy. Similarly, careful palpation of the testes of male rats may lead one to expect to find an increased incidence of interstitial (Leydig) cell tumours of that organ. Notwithstanding these exceptions, the majority of neoplasms and other lesions of the pituitary, thyroid follicular cells, thyroid C-cells, parathyroid gland, adrenal cortex, adrenal medulla, pancreatic islets, ovary and testis remain unsuspected until they

are discovered at necropsy. Perhaps we should be asking ourselves, is this situation as it should be? Why have we not found fit to develop sensitive assay methods whereby we can detect in individual animals, lesions of endocrine glands as they develop? If we had such methods might we not have new and more sensitive tools for investigating the mechanisms of action of pharmaceutical agents, and the mechanisms responsible for the toxicity of environmental agents?

In reality, however, the situation is much worse than has been painted above. When rats and mice, and animals of other species, are killed at the termination of studies, they are subjected to a careful dissection and recording of macroscopic changes. However, whether abnormalities are seen or not, usual practice is simply to submit endocrine tissues like all other tissues to no other than routine fixation, embedding in paraffin wax, and preparation of sections stained only with haematoxylin and eosin. While this sequence of simple procedures will enable one to detect relatively major changes such as focal or generalized hyperplasia or the presence of neoplasms in endocrine tissues, it may be quite inadequate for the purposes of distinguishing between the different cell types that may be involved in these proliferative lesions. For this latter purpose it is sometimes possible by cutting further sections from formalin-fixed, paraffin wax embedded tissues, and applying special stains or immunochemical techniques to them to obtain the additional information one needs. However, in other cases the option to use optimal techniques may have been for ever forfeited by the routine approach to tissue processing.

Irrespective of what it is theoretically possible to do, the fact is that in most studies little or no attempt is made to distinguish the cell of origin of, for instance, pituitary or pancreatic islet cell tumours. In the case of rats it may well be that most tumours of the anterior pituitary consist mainly of prolactin-producing cells. However, eight other kinds of hormone are produced in the gland and it is clear from studies where special techniques have been used that areas of hyperplasia and neoplasia may consist of cells producing hormones other than prolactin or, not infrequently, of mixtures of cells. The question arises, therefore, should we not develop better and more precise techniques for processing and staining endocrine tissues, particularly in the case of the pituitary and pancreatic islets?

All these questions take on a special poignancy when we compare the kind of information about endocrinological disturbances we have for humans with the kind of information about such disturbances we have for animals in long-term toxicity tests. In the case of an individual human we may have, if we think we need it, detailed information on the level of virtually any circulating hormone, and may follow levels of each hormone

of interest sequentially through hours, days, months or years. On the other hand, because necropsy rates are so low, we have very inadequate and inaccurate information about the incidences of neoplasms and foci of hyperplasia of endocrine tissues in humans.

By contrast in experimental animals – more often than not we have little or no information about circulating hormones during the in-life phase of tests and a super-abundance of necropsy data. If the object of toxicological tests is to determine toxicity for safety of test agents for man and to throw light on mechanisms of toxicity, and to alert physicians to potential toxicological – including endocrinological – problems, it really is difficult to imagine that the picture described above is optimal.

Points to be borne in mind by the diagnostician in relation to endocrine toxicology

Many aspects of endocrine toxicology need to be borne in mind by the diagnostician. First, toxic effects on the endocrine system may involve more than one of the many endocrine organs or their target tissues. Secondly, the effects may be manifested as functional and/or structural (pathological) changes and both these kinds of effect may evolve with time. Obviously the diagnostician needs to take both sex and age into account since the sensitivity to toxicants and the manifestations of toxicity are likely to differ with age, and to be different between the sexes. Also, it should never be forgotten that response to endocrine toxicants can be crucially influenced by dietary ingredients (e.g. the iodine content of foodstuffs may modify response to otherwise marginal goitrogens).

In a conventional type of toxicity test, with its structured and relatively standardized set of observations, although the first hints of an effect on the endocrine system often come from treatment-related effects on organ size and weight or from differences in histological appearances of endocrine tissues or their targets, signals from general metabolic tests (e.g. changes in plasma electrolytes, lipid and triglyceride levels, metastatic calcification and cardiac function) should always be carefully considered since they can be indicative of, or associated with, endocrine dysfunction. Also, simple observations of behaviour, reflex responses, and especially of oestrous cycling and mating activity, can be powerful guides to endocrine effects, especially when viewed as part of a pattern.

Complex integrative processes are involved in maintaining homeostasis. When these become disordered as a result of toxicity a wide variety of patterns of disease and pathology may arise. Some signs of disturbed homeostasis are easier to detect than others and presenting signs in humans, who can describe their symptoms, may be different from

those in animals who cannot do so. It is particularly important, therefore, in the evaluation of toxic responses to look for patterns rather than isolated effects, and also to take into account sequences of normal physiological responses. The value of this holistic approach is well illustrated by the consequences and causes of multi-site neoplasia in overfed rodents (e.g. overfeeding predisposes to ageing-related nephropathy which compromises calcium homeostasis and leads to parathyroid hyperplasia, and neoplasia and metastatic calcification).

In our view the most serious problem facing the investigator attempting to interpret observations in long-term toxicity studies in rodents is the need to consider whether an apparent toxic effect should be attributed to the substance being investigated, or whether it is or might be a non-specific consequence of ageing or of disturbance of physiological status. The next section discusses the need for such consideration with particular reference to the effects of overnutrition.

Influence of ageing – particularly ageing secondary to overfeeding – on endocrine status

Before specified pathogen free (SPF) conditions became generally available for toxicological testing the duration of tests was curtailed by morbidity and mortality due to infectious diseases, and infestations by parasites. After the introduction of SPF conditions animals lived longer and the predominant pathology became one of age-related diseases, including, in the case of rats, a wide variety of endocrine disturbances. The spectrum of endocrine disturbances embraced high incidences of neoplasms of endocrine glands, and of hormone-responsive tissues, such as the mammary gland and uterus.

The incidences of endocrine tumours and of other endocrine changes among untreated control rats in carcinogenicity studies in SPF rats are now not frequently such that close to 100% of animals have one or more histologically-evident endocrine neoplasms. Control data reported by Kociba *et al*, (1979) from a two-year study illustrate the point in the case of *ad libitum*-fed Sprague-Dawley rats (see Table 13.1) and our own more recent data do so for *ad libitum*-fed Wistar rats (see Table 13.2).

Such data pose three series of questions. Firstly, does it make any sense to conduct general toxicological tests in animals which towards the end of studies are in such endocrinological disarray as the animals depicted in Tables 13.1 and 13.2? What does it mean if exposure to a test agent is associated with a significant increase or decrease in the incidence of a kind of endocrine tumour which is occurring in high incidence in the untreated control group? Would evidence of a treatment-related change

Table 13.1. *Endocrine tumour incidences in untreated Sprague-Dawley rats (Data from Kociba* et al., *1979)*

		Males	Females
Number of rats observed (%)		86(100)	86(100)
Number of rats with tumours of:			
Adrenal	– cortex	2 (2.3)	6 (7.0)
	– medulla	44(51.2)	7 (8.1)
Ovary	– granulosa cell	–	4 (4.7)
Mammary	– fibroadenoma	1 (1.2)	65(75.6)
	– cystfibroadenoma	–	11(12.8)
	– adenoma	–	10(11.6)
	– fibroma	–	4 (4.7)
	– adenocarcinoma	2 (2.3)	7 (8.1)
Pancreas	– islet cell	14(16.3)	8 (9.3)
Parathyroid		–	1 (1.2)
Pituitary	– anterior lobe	29(33.7)	54(62.8)
Testis	– Leydig cell	4 (4.7)	–
Thyroid	– C-cell	7 (8.1)	7 (8.1)

in the incidence of a kind of endocrine tumour which is found in between 10 and 60% of untreated control animals have any toxicological significance for man? In circumstances where an endocrine tumour is occurring in high incidence in untreated control animals, should treatment-related changes in the incidence of tumours that fall into this category be used in the assessment of the test compound for carcinogenicity?

Secondly, can one assume that abnormality in endocrine status has no effect on the manifestation of other forms of toxicity? Many manifestations of toxicity in organs such as the liver and kidney differ in incidence and severity between males and females and are known to be influenced by castration and/or the administration of sex hormones.

Thirdly, although the more obvious disturbances of endocrine status in *ad libitum*-fed rats are most clearly seen at the termination of studies of two years or longer duration, it is clear that the beginnings of these changes take place much earlier in life. Foci of hyperplasia in the pituitary gland are already evident in *ad libitum*-fed rats of less than one year old, and prolactin levels well above the physiological range begin to be evident from the age of about six months onwards. In addition to this, irregularity of oestrus cycling is frequently rife in *ad libitum*-fed SPF female rats aged only about one year. Apart from its being obvious that rats showing such evidence of endocrine disturbance during middle life are not appropriate models for endocrinologically-normal humans, there

Table 13.2. *Percentage life-time incidence of certain endocrine tumours (benign and/or malignant) in* ad libitum*-fed Wistar rats in a study of 30-months duration*

	Males		Females	
	AL	80% of AL[1]	AL	80% of AL[1]
Number of rats	100	100	100	100
% rats with tumours of:				
Adrenal				
– cortex	0	1	2	3
– medulla	3	4	3	1
Ovary	–	–	4	3
Mammary	0	0	37	9**
Pancreas				
– islet cell	10	1*	2	1
Parathyroid	5	1	2	1
Pituitary				
– anterior lobe	30	14**	62	46*
– intermediate lobe	13	9	6	0
Testis				
– Leydig cell	23	30	–	–
Thyroid				
– Follicular	1	1	2	0
– C-cell	2	2	8	4

AL = ad libitum; * = $p<0.01$; ** = $p<0.001$

[1]Survival was highly significantly better in the 80% of AL animals than in the AL animals hence the beneficial effects of diet restriction were actually greater than those shown and none of the apparently adverse effects are real.

is the fact that the manifestations of endocrine abnormality vary widely from rat to rat. And yet, as pointed out above, the design of studies is such that no attempt is made during the in-life phase of experiments to assess the endocrine status of individual animals.

There is abundant published evidence that, in rats, calorie-restriction prolongs life, and reduces the age-standardized incidences of many different ageing-related non-neoplastic and neoplastic diseases. Notable among the non-neoplastic conditions affected are chronic progressive nephropathy, polyarteritis, acute and chronic prostatitis, mammary gland hyperplasia, secretory activity and galactocoele formation, and a range of inflammatory conditions of the skin and subcutis. Severe chronic nephropathy impairs the maintenance of mineral balance with regard

especially to calcium, magnesium and phosphate. A consequence of this impairment is hyperplasia and neoplasia of the parathyroid gland. A second consequence is an increased risk of hyperplasia and neoplasia of the adrenal medulla (Roe & Baer, 1985). Under conditions of slight dietary restriction, the incidence of parathyroid and adrenal medullary proliferative conditions tend to be significantly less. It is, however, not only the fact that overfeeding greatly increases the risk of development of ageing-related kidney disease which predisposes to parathyroid and adrenal disease, which gives cause for concern. There is also the fact that the effects vary widely from animal to animal and no attempt is being made during the in-life phase of studies to assess individual animals for the severity of nephropathy.

Obviously there exists a strong case for conducting all chronic toxicity and carcinogenicity experiments under conditions wherein premature ageing does not occur and does not introduce unwanted between-animal variation in endocrine status, and it is clear that one can partly achieve this by conducting tests under conditions of diet restriction. However, there are those who argue that dietary restriction does no more than postpone the evil day and that eventually, at an albeit later age, calorie-restricted rats end up with the same spectrum of ageing-related diseases, and in the same high incidence as *ad libitum*-fed rats. This is, in fact, not wholly true. The lifetime expectation of developing several conditions is in fact lower in calorie-restricted rats. Another commonly put forward argument is that carcinogenicity tests would have to be continued for longer than in *ad libitum*-fed animals. This, it is claimed, would simply increase the costs without any obvious benefit. We would argue, however, that experiments should not be continued after animals have begun to be in endocrinological disarray due to ageing. Experiments should be terminated while control rats are still in normal physiological status, that is, while it is still reasonable to claim that they are likely to be appropriate models for man.

The last point that we need to make here is that calorie-restriction is not a panacea for all the problems associated with the use of laboratory rats and mice in chronic toxicity and carcinogenicity tests. Although calorie restriction reduces the incidence of endocrine disturbances it by no means abolishes them. As illustrated in Table 13.2 tumours of the anterior lobe of the pituitary and of the testis may still be very high in calorie-restricted rats. This problem has been evident for years and yet no serious attempt has been made to overcome it. It is, of course, possible that genetic constitution is responsible for these unhuman-like characteristics. If so, then we need to be bold enough to abandon the strains we are using and start again to develop strains which are free from such genetic

flaws. Alternatively, it is possible that the high incidences of endocrine changes which we are seeing, even in diet-restricted rats, are due to environmental factors which we as yet do not understand and cannot define. We would argue that complacency with regard to the adequacy of currently used animal models is misplaced and that there is an urgent need for fundamental research aimed at developing animal models that are more reliable, particularly from an endocrinological standpoint.

Quantification of effects on endocrine status

When it is reasonably certain in a toxicological study that there has been an effect on endocrine status, it may be necessary to quantify it. If the mechanism involved is unclear further *in vivo* studies may be needed to elucidate it (e.g. further *in vivo* testing will be needed to explore hypothalamic-Leydig cell performance and adrenalcortico-steroids in a case presenting as simple virilization). On the other hand, if the mechanisms involved are known, quantification may sometimes be achieved by the use of well-established *in vitro* diagnostic and assay procedures (e.g. tissue culture methods are available for studying the effects of secretagogues and membrane transport inhibitors of iodine uptake and metabolism by thyroid cells).

In no other area of toxicology is it more important than in endocrine toxicology to establish the specificity and sensitivity of methods used for measurement. The scientific literature is full of examples of erroneous conclusions being drawn in studies where non-specific and/or insufficiently sensitive methods have been used.

Extrapolation from animals to man

The basic principles underlying the prediction from the findings in laboratory studies of risk for man (or for some other species in the case of veterinary products) are common to all areas of toxicology. They involve, *inter alia*, obtaining answers to four questions. First: 'Are the test species and man physiologically similar in relation to the function and activity of the endocrine gland(s) involved?' Secondly: 'Are the circumstances of exposure (i.e. dose and duration) to the test substance and its active metabolites, particularly at the target site(s), similar in man and the test species?' Thirdly: 'What is the mechanism of action in the test species: is it direct or indirect and would it be operative in man?' Lastly: 'If knowledge is lacking, is it possible, ethically, to determine whether the endocrine targets in the test animals are, in fact, targets in man?'

If the answers to all these questions are in the affirmative, the predic-

tion of risk for humans may be straightforward. Often, however, variability of endocrine responses in different circumstances tends to engender uncertainty so that precise extrapolation is impossible and great caution is necessary.

Perhaps the most difficult areas in which to make a risk assessment are those in which the function and importance of a hormone and its consequential responses vary from one species to another (e.g. somatotrophin and prolactin in rodents versus humans), or in which the normal role of a hormone is uncertain (e.g. many neuropeptides in the gastrointestinal tract). Risk assessment is also difficult where it seems that the same hormone can have different effects at different sites (e.g. cholecystokinin and vasoactive intestinal peptide in the peripheral versus the central nervous systems), or where little or nothing is known of the mechanism of action of a hormone.

It may also be very difficult to evaluate properly the toxicological significance of tumours of the endocrine system that are endocrinologically silent (e.g. many chromophobe adenomas of the pituitary and adrenal cortical adenomas), or which do not appear to have been caused by conventional hormonal stimuli (e.g. adrenal medullary tumours secondary to disturbance of calcium homeostasis). Neoplasms of the diffuse neuroendocrine system are likely to fall into this class, at least until more is known about their pathogenesis and the physiological roles of their products.

Future possibilities and uncertainties

Rapidly increasing knowledge in the fields of general receptorology, membrane receptors and channels, signal transduction mechanisms, and various areas of molecular biochemistry are impacting importantly on the understanding of mechanisms in endocrinology. These advances have been paralleled by greater understanding of the sources and nature of various dynamic integrative controlling systems. Even more exciting are recent discoveries of interactions between DNA and hormones and of how these may sometimes be subverted (e.g. the very recent identification of the possible receptor for peroxisome proliferation in the liver as a member of the oestrogen receptor super family). In this area, clearer understanding will surely lead to an ability to predict sites where xenobiotics may act to affect endocrine status.

At a different level, the discovery of endocrine, paracrine and autocrine controls on cell proliferation has not only enlightened our understanding of carcinogenesis, it has also brought the concepts of

endocrinology into a broader area of pathology – i.e. neoplasia. That, in turn, emphasizes the importance of understanding of how normally responsive cells may become abnormal, insensitive, autochthonous tumour cells (e.g. the selective loss of control by insulin-like growth factor-1 in thyroid cells during at least one particular type of thyroid carcinogenesis).

Many uncertainties stem from the carrying out of very detailed and precise investigations on what are, in fact, nothing more than laboratory artefacts. Every investigator using model systems which are several stages away from real life should constantly be asking himself whether what he is observing relates to real life, or only to the model. If reliable micro methods requiring only very small samples of blood existed for the monitoring of circulating hormones in routine toxicity studies, they might be of great help in mapping out the pathogenesis of lesions which presently remain unsuspected until necropsy is carried out. At present, however, studies of endocrine effects is not an integral part of toxicological assessment, so that research on suspected endocrine effects has to be undertaken in separate and often quite costly studies. Clearly, careful thought combined with a full evaluation of chemical structure, known pharmacological activity, and the results of other toxicological studies, need to be given before special studies are contemplated.

An area that has not received much attention, and which merits further study, is the involvement of the endocrine system in toxicity. At a simple level, enzyme induction in the liver, which involves massive synthesis of proteins, requires normal somatotrophin, insulin and thyröxine levels for its full expression. How might it respond to limited abnormality of even just one of these factors? Similarly, renal clearance of many materials is sensitive to circulatory changes produced by a variety of peptide hormones. Other examples include the effect of androgens in inducing 2μ globulin synthesis in the rat, and the partial permissive role of sex hormones in determining the response of sensitive epithelia to carcinogens, etc.

Overall, endocrinology seems well suited to toxicological experimentation, because its actions can be quantified and mechanisms can be explored, provided that a signal action is identifiable. An orderly progression of investigative studies can often be envisaged, extending from first detection of an effect in a simple experiment to precise mechanistic analysis at the molecular level. It has been argued that toxicology is no more than the flipside of pharmacology. What could be more satisfying to a toxicologist than to deduce from toxicological observations how a regulatory hormone works?

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