

*Meeting Report***Antagonists for gluco- and mineralo-corticoids**

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Antagonists of various hormonal steroids are important not only as molecular probes to delineate adreno-corticoid action but also as potential drugs in syndromes of hormone excess and in control of fertility. Although various levels of hormone antagonism are possible, the major emphasis of this workshop was to review recent advances in the chemical synthesis and biological activity of molecules capable of acting at the level of the specific receptor in the cell. Other means of antagonism were reviewed as well in order to provide an opportunity for interaction between experts in different fields.

Dr Sonino (Padova) reviewed the inhibition of steroid hormone synthesis and clinical management of hormone excess syndromes, primarily Cushing's disease, by aminogluthemide, metyrapone and o'p'DDD, where side effects pose serious problems. Immunization of rats with corticosterone was attempted by Dr Vecsei (Heidelberg) with some success but it appears some way off before it can be exploited as a specific means to antagonize hormone activity.

Dr Duax (Buffalo) analysed X-ray crystal conformations of a number of molecules to identify the structural features associated with receptor binding and biological response. Although aldosterone has 7 possible structural isomers, the most stable form remains the 18,11-acetal-20,18-

hemiketal isomer in which the A ring of the steroid is drawn toward the  $\beta$ -face as a result of epoxide formation. The only common feature between aldosterone and its antagonists spironolactone and canrenone is the 4-en-3-one composition and the conformation of their A-rings; the absence of hydrogen donating groups in the D-ring may account for the antagonist activity of the latter two molecules.

18-Deoxyaldosterone possesses one third the binding affinity of aldosterone for the cytoplasmic receptor but exhibits a 2:1 antagonist to agonist ratio, despite close resemblance in the shape of rings A, B, C, and E.

Dexamethasone oxetanone is a potent glucocorticoid antagonist but its A, B and C rings are nearly identical to that of dexamethasone, the agonist. However, although both molecules can accept a hydrogen bond at O(20), only the agonist can donate two hydrogen bonds in the D-ring.

Collectively, high affinity binding to the mineralocorticoid receptor (MR) appears to be related to a complementary fit between amino acids of the receptor site and a flat 4-en-3-one A ring; the glucocorticoid receptor (GR) appears to prefer a 4-en-3-one A ring that is bowed towards the  $\alpha$ -face. Specific interactions between the steroid B, C and D rings and the receptor appear to play at best a minor role in receptor binding but

are the most important factor in determining agonist vs antagonist behaviour subsequent to binding.

Dr Wambach (Cologne) explored structure-activity correlation of newly synthesized aldosterone antagonists. Replacement of the 17-spirolactone ring by a  $17\alpha$ -hydroxypropyl or a  $17\beta$ -hydroxyl group led to a loss of affinity for the receptor without a loss of antialdosterone action in vivo. C6/C7 unsaturated compounds, and methylation of the D-ring of spirolactone reduced activity both in vivo and in vitro. Substitution of the  $7\alpha$ -thioacetyl group in the  $\beta$ -position (prorenone) increased activity in vivo as well as in vitro. Two derivatives of spirorenone were 3–8 times more active than spirolactone in vivo but their affinity for the MR was only slightly increased. If spirolactone was taken as 100%, the relative affinity of  $6\beta$ -,  $7\beta$ -,  $15\beta$ -,  $16\beta$ -dimethylene derivatives of spironolactone for MR ranges from 0.1–200%.

The clinical usefulness of spironolactone is limited by its antitestosterone properties, as evident by binding to the androgen receptor (AR) in prostate cytosol. Although the affinities for the MR and AR do not correlate derivatives with higher affinity for AR than spironolactone are more potent than this molecule for testosterone receptors in vitro.

Dr Ramsay (Sheffield) pointed out that new spirolactones are clearly needed for treatment of essential hypertension and oedematous states since molecules now available provoke gynaecomastia and other side effects. In vitro binding assays of newly synthesized derivatives did not provide a reliable means of predicting their potency in man, and could not distinguish agonists from antagonists. Prediction of renal potency in man was improved when lactones were used in binding studies in place of carboxylic acids. Furthermore, metabolic transformation of spirolactones was extensive, complex and widely different between various species. It was suggested that newly synthesized materials should be tested for binding to the MR in vitro and then tried clinically at the earliest possible stage.

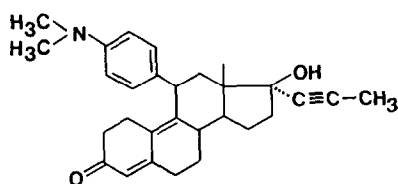
Dr Sandor (Montréal) attempted a comparison of the binding properties of GR and MR from various species. In the domestic duck and chicken, the most powerful GR agonists (corticosterone,

progesterone) antagonize aldosterone binding to the putative MR in the intestinal mucosa. A close similarity in the hydrodynamic properties of GR and MR was noted. In the fish, cortisol antagonizes corticosterone binding to GR whereas progesterone and aldosterone show no GR affinity. There are clear hydrodynamic differences in the GR from these two sources (avian vs fish). He suggested that in the bird, the same macromolecule adopts a conformation of either GR or MR, depending upon the agonist. In the vertebrate, however, GR appears to be an ancestral whose MR functions evolved through interaction with the tetrapod hormone aldosterone.

Dr Teutsch (Romainville) reported a major breakthrough in the search for anti-glucocorticoids by a method developed at Roussel (Patent 2213272). This epoxide pathway consists of epoxidation with hexafluoroacetone hydroperoxide, or 30% hydrogen peroxide-hexachloroacetone in the  $11\beta$  position of 19-nor-steroids followed by a conjugate opening of the epoxide by lithium cuprate to the  $11\beta$ -substituted compound as the sole reaction product. This is by far the most convenient method for introducing almost any organic group to yield  $11\beta$ -substituted 4,9-estradienes and 1,3,5(10)-estratrienes.  $11\beta$ -substitution with vinylic or aromatic radicals led to very high binding affinities for both the GR and the progestin receptor (PR), both of which possess a large hydrophobic pocket of 10–12 Å able to accommodate substituents as large as a phenyl ring. Furthermore, the presence of a hydrogen bond donor in the steroid clearly did not appear necessary for binding to the receptor. It was established that mere C-11 substitution can transform an agonist into an antagonist. Thus, the  $11\beta$ -vinyl derivative (RU 42764) is a full agonist but the  $11\beta$ -4-dimethylaminophenyl compound (RU 38486) is a pure antagonist, both in the glucocorticoid and the progestational sense. Aliphatic substitutions in the  $11\beta$  position are compatible with GR binding while considerably decreasing affinity for PR. However, complete dissociation of GR from PR has not yet been possible. Notwithstanding, a major breakthrough in the anti-glucocorticoid field has been attained.

Dr Philibert (Romainville) reviewed for the first time the biological activity of RU 38486 [17 $\beta$ -hydroxy- $11\beta$ -(4-dimethylaminophenyl)-17 $\alpha$ -(1-propynyl)-estra-4,9-diene-3-one], shown below. Its

affinity for both rabbit uterine PR and rat thymus GR was 5 times that of progesterone and 3 times that of dexamethasone. In thymocytes, it fully antagonized the thymolytic effects of dexamethasone in concentrations lower than that of the agonist on total organ weight and RNA synthesis. In vivo, a single oral dose of 10 mg/kg totally inhibited induction of rat liver tryptophan pyrrolase, tyrosine transaminase and glycogen in response to 10  $\mu$ g/kg dexamethasone, and also blocked the action of the glucocorticoid on ACTH secretion and diuresis. In all bioassays it was totally devoid of any agonist activity up to  $10^{-6}$  M in vitro and 100 mg/kg in vivo.



RU 38486

RU 38486 is also a potent antiprogesterative with inhibition of endometrial proliferation in rabbits and antinidatory and abortive properties in the rat. Antiandrogenic property on total rat prostate mass was noted at dose level of 30 mg/kg, orally. Clinically, it has already undergone successful trials in the treatment of Cushing's syndrome, and in termination of unwanted pregnancy, both without side effects. Thus, a revolutionary new material is clearly available for the first time.

Dr Agarwal (Paris) pointed out that RU 38486 reversed the protective effect of dexamethasone against endotoxin shock and also sensitized mice to a sublethal dose of the toxin. Again, no agonist activity of RU 38486 was noted, although the time course of action suggested that GR may not be directly involved under these conditions.

Dr Berry (Austin) described the mechanism of action of the glucocorticoid antagonising factor (GAF) which is released from the macrophages of animals given bacterial endotoxins that are well known to oppose hormone induction of liver enzymes in vivo. GAF did not interfere with the formation or activation of the hormone-receptor complex. However, GAF interferes with the transcription of the gene that codes for liver

phosphoenolpyruvate carboxykinase in some unknown way. Being an antagonist of endogenous origin, GAF may find an eventual clinical application but large scale production and purification are still a way off.

Dr Moudgil (Rochester) reported that the binding of steroid-receptor complexes to isolated nuclei, DNA-cellulose and ATP-Sepharose was abolished if the complex is incubated at 23°C in the presence of molybdate, tungstate or vanadate. Pyridoxal 5'-phosphate, aurintricarboxylic acid, heparin, *o*-phenanthroline and rifamycin AF/103 completely blocked the binding of the receptor to the acceptor either by inhibition of receptor activation or by decreasing the affinity of the receptor for the acceptor. The mechanism of action of these inhibitors appears to be different and may be exploited in receptor purification.

Dr Sato (Osaka) reported the presence of small  $M_r$  dialysable materials that inhibit rat liver GR activation in vitro. This was not true of rat uterine estrogen receptor (ER). Dr Kalimi (Richmond) could remove factors of this type by dextran charcoal leading to diminished dexamethasone binding. His factor is not dialysable, thermolabile, had a Stoke's radius of 4–5 nm, and was inactivated by RNase and dithiothreitol. Calcium and magnesium ions, too, accelerated GR inactivation whereas EDTA and EGTA stabilized receptor binding. Glucocorticoid antagonism was also exhibited by several intracellular enzymes (phospholipases, proteases, alkaline phosphatases). The role of all these substances, that occur naturally in vivo, needs careful scrutiny to delineate hormone action.

It is now well accepted that the receptor for all classes of steroid hormones is heterogeneous. This heterogeneity manifests itself in various ways depending upon the tissue, the steroid, and the resin used for receptor fractionation. Despite this obvious polymorphism, almost invariably it is assumed that a 'classical' receptor must be hidden somewhere in the midst of all this multiplicity of steroid-bound receptor peaks. Instead of just shrugging off this mass of biochemical data, Dr Agarwal (Paris) attempted a physiological interpretation of receptor heterogeneity. To date, it has not been possible to establish whether these multiple peaks are indeed different proteins or modified versions of a more fundamental, basic unit. It is however inconceivable that the genetic repertory

should possess sufficient flexibility to transcribe separate receptors for all possible synthetic agonists and antagonists that are being made available day after day. It is inconceivable, too, that all agonists of mineralocorticoids, for example, should exert a physiological action by saturating the same site, since deoxycorticosterone and its 18-hydroxy derivative possess only a tenth of the affinity of aldosterone for MR and are produced in only trace amounts *in vivo*.

Some of the confusion stems from the fact that receptor is quantitated only after it has interacted with the hormone. The nature of the native receptor *in vivo* appears to be quite different. First, receptor stabilization by steroid hormones is well established but formation of this complex, and subsequent activation, leads to nuclear transfer. Heat activation *in vitro* has no relevance *in vivo* where all processes are simultaneously occurring at 37°C or so. Second, adrenalectomy depletes endogenous hormones but actually increases the amount of available receptor *in vivo* – in contradiction to the stabilizing role of the hormone in

*vitro*. Thus, the inescapable conclusion is that the presence of free receptor in cell cytoplasm *in vivo* must be due to mechanisms that cannot be explained by observations that have hitherto been made *in vitro*.

As shown in fig.1 it therefore seems very likely that a nascent, proreceptor is consistently present in the cytoplasm, is not bound to the steroid, and its stability is assured by its association with the ribosome after translation of the receptor mRNA. As soon as an appropriate ligand is presented to this nucleoprotein complex, the receptor protein may be freed of its ribosomal support. Rat liver GR is known to be associated with RNA and RNase A converts it from a heavy 7–8 S to a light 3–4 S form that is conventionally assayed *in vitro*.

Thus, a basic tenet of the model presented here is the right conformation conferred upon the ribosome-bound proreceptor by the ligand, thereby rendering it geometrically disposed for post-translational modification by a whole battery of ions, chelators, inhibitors and activators (fig.1). The levels of receptor *in vivo* are regulated by the

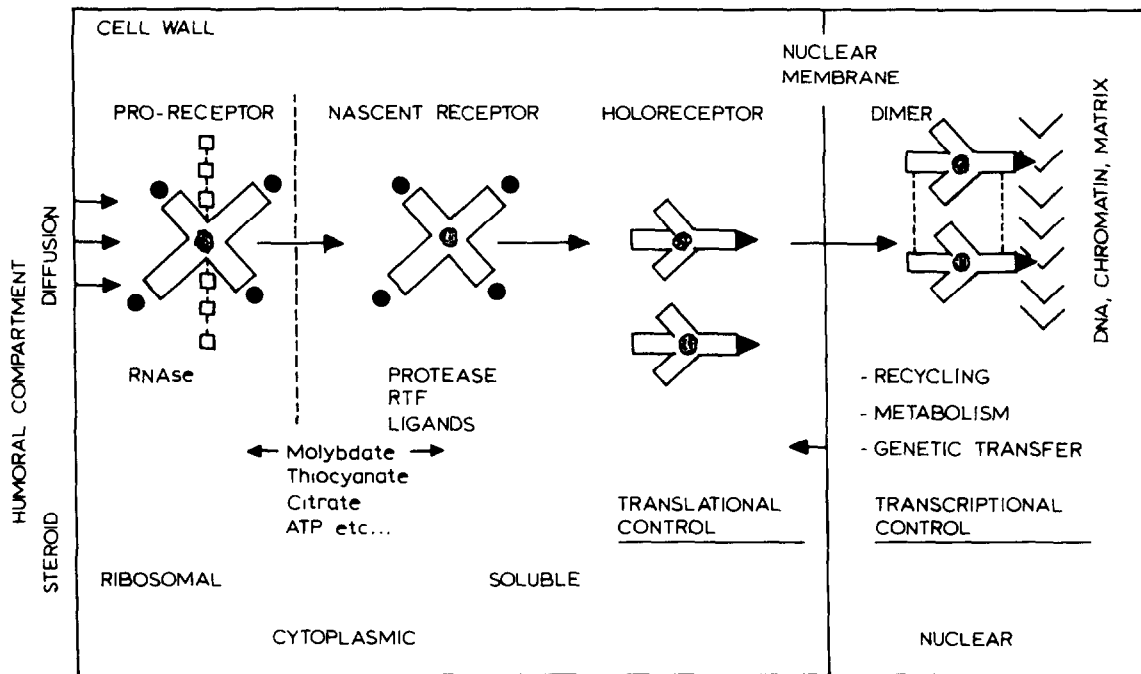


Fig.1. The Argusoid model. The name Argusoid is derived from the Greek myth of Argus, whose chief role was the surveillance and coordination by virtue of 100 eyes, just as the receptor must be sensitive to a myriad cellular demands, processes, and modulating factors.

amount of the specific ligand and support the model here. In addition, the mature holoreceptor may be fully active at the level of MTV translation, contrary to the classical viewpoint where the steroid-receptor complex modulated only gene transcription.

It follows, therefore, that exploitable targets for hormone antagonism may be sought at all of the levels shown in the model, although such efforts are usually limited to steroid-receptor-acceptor association *in vitro*.

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