istered in 0.1 mL of water or 5% $NaHCO_3$, and AI and AII pressor responses were evaluated for up to 70 min. For oral testing, compounds were administered in 0.75 mL of water, 5% $NaHCO_3$, or 1% agar suspension and AI and AII pressor responses were evaluated for up to 280 min. Maximum percent inhibition was determined as the mean of the responses for four animals per dose.

Inhibition of Angiotensin I Induced Pressor Response, 24 h Predosing. Male Sprague-Dawley rats were prepared as described for the experiments above. The rats were fasted for 24 h before and 2 h after the oral administration of water (control group) or drug. Twenty-four hours after drug administration, each rat was given AI (310 ng/kg), and the pressor response was recorded as described above. The mean of the responses for each drug-treated group were compared to the responses of groups given only water.

Registry No. **5**, 13500-53-3; **6b** (free acid), 13504-86-4; **6d** (free acid), 75176-20-4; **6d** (methyl ester), 75176-19-1; **6d**·CHA, 75176-21-5; **6g** (free acid), 75776-49-7; **6g** (methyl ester), 16217-17-7; **6g**·CHA, 75776-50-0; **6h**·CHA, 113949-39-6; **6i**·CHA, 113949-41-0; **6j** (free acid), 83552-11-8; **6j**·CHA, 83552-12-9; **6k**·CHA, 75176-29-3; **6m** (free acid), 94898-97-2; **6m**·$AdNH_2$, 94898-98-3; **6p**·CHA, 81806-15-7; **6g** (free acid), 75776-77-1; **6g** (methyl ester), 75776-76-0; **6q**·CHA, 75776-78-2; **6a** (free acid), 78464-03-6; **6u** (lactone), 113949-45-4; **6u**·$AdNH_2$, 78464-14-9; **6v** (free acid), 82087-66-9; (*E*)-**6z** (free acid), 114029-40-2; (*Z*)-**6z** (free acid), 114029-41-3; (*E*)-**6z**·DCHA, 114029-42-4; (*Z*)-**6z**·DCHA, 114029-43-5; **6aa** (free acid), 64187-47-9; **6bb** (free acid), 75776-55-5; **6bb** (methyl ester), 75776-54-4; **6bb**·CHA, 75785-37-4; **7d** (free acid), 75176-22-6; **7e** (free acid), 113949-37-4; **7g** (free acid), 75776-51-1; **7h**·HCl, 113949-42-1; **7i**·HCl, 83552-27-6; **7j**·HCl, 83552-13-0; **7k**·HBr, 105107-84-4; **7k** (*N*-BOC derivative), 83623-88-5; **7m**·HBr, 94898-99-4; **7p**·HBr, 81806-16-8; **7q**·HCl, 83552-41-4; **7s**·HCl, 82087-67-0; *trans*-**7t** (free acid), 96314-26-0; *cis*-**7t** (free acid), 103290-40-0; **7w**·HCl, 82087-68-1; **7x**·HCl, 90657-55-9; **7y** (free acid), 82087-73-8; **7bb** (free acid), 75776-56-6; **8a**, 57653-38-0; **8b**, 77345-53-0; **9i**·HCl, 83552-28-7; **9j**·HCl, 83552-14-1; **9k**·HCl, 83552-04-9; **9q**·HCl, 83552-42-5; **9s**·HCl, 83552-24-3; **10k**, 113949-43-2; **10k** (BOC-deblocked), 113949-53-4; **10q**, 113949-44-3; **11**, 74345-73-6; **12**, 74654-91-4; **13a**·DCHA, 114127-21-8; **13b** (free acid), 114029-44-6; **13c**·DCHA, 75197-32-9; **13d**·DCHA, 75246-74-1; **13e** (free acid), 113949-46-5; **13g**·DCHA, 75785-36-3; **13h**·DCHA, 113975-20-5; **13k** (free acid), 75176-36-2; **13k**·DCHA, 75197-36-3; **13l**·DCHA (diastereomer 1), 114029-50-4; **13l**·DCHA (diastereomer 2), 114029-52-6; **13m**·DCHA, 94899-01-1; **13p**·DCHA, 81814-77-9; **13q**·DCHA, 75776-81-7; **13r**·DCHA, 113949-48-7; **13s**-DCHA, 82165-74-0; **13w**·DCHA, 82092-95-3; **13y**·DCHA, 82092-98-6; **13bb**·DCHA, 75785-38-5; **14a** (free acid), 113564-51-5; **14b** (free acid), 113564-58-2; **14c** (free acid), 75176-14-6; **14d** (free acid), 75197-34-1; **14e** (free acid), 113949-49-8; **14f** (free acid), 81814-74-6; **14f** (methyl ester, *S*-benzoyl derivative), 81814-75-7; **14f**·$AdNH_2$, 81872-08-4; **14g** (free acid), 75776-53-3; **14h** (free acid), 113564-63-9; **14h**·$AdNH_2$, 114029-46-8; **14k** (free acid), 75176-37-3; **14k** (methyl ester, *S*-benzoyl derivative), 113949-52-3; **14k**·$AdNH_2$, 114029-53-7; **14k**·Arg, 81872-09-5; **14l** (free acid, diastereomer 1), 114029-45-7; **14l** (free acid, diastereomer 2), 114029-55-9; **14m** (free acid), 94899-02-2; **14n** (free acid), 81814-88-2; **14n**-Arg, 81872-11-9; **14o** (free acid), 81814-64-4; **14o**·Arg, 81814-65-5; **14p** (free acid), 81814-78-0; **14q** (free acid), 75776-82-8; **14r** (free acid), 113949-50-1; **14r**·$AdNH_2$, 113949-51-2; **14s** (free acid), 82092-93-1; **14s**·$AdNH_2$, 114029-47-9; **14s**·Arg, 114029-48-0; **14w** (free acid), 82092-96-4; **14w**·Arg, 82165-75-1; **14y** (free acid), 82092-99-7; **14aa** (free acid), 77282-50-9; **14aa**·$AdNH_2$, 77282-51-0; **14bb** (free acid), 75802-72-1; **15k** (free acid), 81872-10-8; **15k**·$^1/_2Ca$, 81938-43-4; **15k**·k, 81938-42-3; **16a**, 80586-33-0; **16a** (free acid), 81814-84-8; **17**, 82717-96-2; **18i**, 83552-30-1; **18j**, 83602-04-4; **18k**, 83552-06-1; **18k** (ethyl methyl ester), 83552-05-0; **18q**, 83602-05-5; **18s**, 83552-23-2; **18w**, 83552-26-5; **19k** (free acid), 83551-92-2; **19k** (trimethylsilyl)ethyl ester, 113949-54-5; **19k**·HCl, 114029-54-8; **19q** (free acid), 83647-97-6; **20**, 83623-46-5; **21**, 83623-61-4; **22c** (free acid), 113949-61-4; **22c**·2Li, 113949-60-3; **22d** (free acid), 113949-62-5; **22d**·2Li, 83624-12-8; **22h** (free acid), 83624-60-6; **22i** (free acid), 83745-60-2; **22j** (free acid), 113949-57-8; **22k**·2Li, 113949-58-9; **22q** (free acid), 83623-49-8; **22q** (methyl ester), 83623-48-7; **22q**·2Li, 83623-50-1; **22s** (free acid), 83624-30-0; **22t** (free acid), 113949-56-7; **22t** (*P*-ethyl ester), 113949-55-6; **22w** (free acid), 83624-20-8; **22x** (free acid), 95399-71-6; **22x**·2Li, 113975-21-6; **22y**·2Li, 113949-59-0; ACE, 9015-82-1; PhOH, 108-95-2; PhSH, 108-98-5; MeSH, 74-93-1; $HOCH_2CH_2OH$, 107-21-1; $HSCH_2CH_2SH$, 540-63-6; $Me_3SiCH_2CH_2OH$, 2916-68-9; $PhCH_2P^+Ph_3 \cdot Cl^-$, 1100-88-5; *N*-(benzyloxycarbonyl)-*cis*-4-phenoxy-L-proline benzyl ester, 113949-36-3; 2-naphthalenethiol, 91-60-1; 2-naphthol, 135-19-3; *N*-(phenylthio)succinimide, 14204-24-1; *N*-(*P*-chlorophenylthio)succinimide, 42839-20-3; *N*-(*P*-fluorophenylthio)succinimide, 779-73-7; *trans*-4-hydroxy-L-proline, 51-35-4.

Supplementary Material Available: Structure factor tables for compound **7t** (2 pages). Ordering information is given on any current masthead page.

Binding of Steroids to the Progestin and Glucocorticoid Receptors Analyzed by Correspondence Analysis

Tiiu Ojasoo,*† Jean-Christophe Doré,‡ Jacques Gilbert,‡ and Jean-Pierre Raynaud†

Roussel-Uclaf, 75007 Paris, France, CNRS UA 401, Muséum National d'Histoire Naturelle, 75005 Paris, France, and CERCOA-CNRS, 94320 Thiais, France. *Received August 3, 1987*

The relative binding affinities of over 30 steroids have been measured for the cytosol glucocorticoid receptor (GR) of thymus, liver, and hepatoma tissue culture cells and for progestin, androgen, and mineralocorticoid receptors. The data have been analyzed by correspondence analysis to reveal the singularities among the receptors of different hormonal classes, the similarities in GR of different origins, and the different specificities of the ligands. Additional data on new steroids have been injected into the system as well as results on a further parameter, namely the induction of tyrosine aminotransferase (TAT) activity, to illustrate the power and flexibility of the methodology. The analysis has confirmed previous correlations between GR binding and TAT response but also highlighted the antiglucocorticoid activity of progestins. This method should prove to be a substantial aid to the interpretation of increasingly complex data, in particular with regard to the action of existing and newly synthesized steroids on glucocorticoid systems of differential sensitivity.

In the early 1970s we set up a screening system for measuring the relative binding affinities (RBAs) of test steroids to the estrogen (ER), progestin (PR), androgen (AR), glucocorticoid (GR), and mineralocorticoid (MR) receptors. This system yields two types of information: first, whether a steroid can recognize one or more receptors (specificity profile) and second, from data measured at short and long incubation times, whether interaction kinetics are faster or slower than for reference hormones.[1-3]

* To whom correspondence should be addressed at Roussel-Uclaf, 35, bd des Invalides, 75007 Paris, France.
† Roussel-Uclaf.
‡ CNRS UA 401 and CERCOA-CNRS.

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Biologically active progestins are mostly either derivatives of progesterone (pregnane derivatives) or of ethynylnortestosterone (norandrostane derivatives). Analysis of screening data has shown that several potent progestins (e.g., norethisterone, norgestrel) can bind with relatively slow interaction kinetics not only to PR but also to AR. A systematic study of the importance of several structural features in their binding to AR has already been published following analysis of the RBA data by correspondence analysis.[4]

The screening data, however, also suggested that there is a more general relationship between competition for binding to PR and GR. The majority of progestins would appear to form a fast-dissociating complex with GR that can result in antiglucocorticoid activity.[5,6] Furthermore, it has recently been confirmed that progestins can accelerate the dissociation of labeled dexamethasone from GR, thus affording a further explanation for antagonist activity.[7-9]

These results imply a certain degree of homology in the ligand-binding domains of PR and GR. There are many similarities between these two receptor proteins in other respects: they have similar gross physicochemical characteristics (e.g., sedimentation properties),[10,11] they are both associated with a 90K Da nonsteroid-binding phosphoprotein when in an untransformed state stabilized by molybdate,[12,13] they have at least one common antigenic determinant,[14] but other antibodies raised against one receptor did not cross-react with the other,[15,16] and they can bind to the same sites in two hormonally regulated promoters but recognize distinct features of these elements.[17] Presently, cloning of the receptor cDNAs is revealing the detailed sequence homologies whether in the DNA or ligand binding domains.[18-26] However, until crystals of the receptor proteins are available, the 3D structure of the steroid binding sites will have to be predicted by molecular modeling techniques with all the limitations that these entail. It therefore remains of crucial interest to analyze in depth the subtle variations in binding specificity resulting from structural modifications of the effector steroids (changes in H-bonding, van der Waal's surfaces, conformation, etc.). Each ligand may stamp an individual conformation on the receptor protein. Recently, the presence of isoforms of PR with different molecular size and shape have been reported as a function of the radioligand employed.[27]

In the present paper, we have attempted to interpret as meaningfully as possible existing data on the progestin and glucocorticoid binding of a series of progestational and glucocorticoid drugs as well as of new derivatives. Binding to PR was measured on rabbit uterus cytosol, binding to GR on cytosol from rat thymus, liver, and hepatoma tissue cells in culture (HTC cells), and correlated with agonist and antagonist activity on the induction of tyrosine aminotransferase (TAT). This analysis should contribute toward the identification of specific or interchangeable structural discriminants for binding to PR and GR and toward our understanding of the similarities in specificity among GRs from different tissues. If performed on a sufficiently vast number of varied molecules, it could constitute valuable guidance for the design of new tailor-made ligands.

In order to avoid foregone conclusions on the importance of known features of the molecules, the matrix of steroid RBA data relating to different classes of hormone receptor (PR, GR, AR, MR) from different sources (thymus, liver, HTC) was analyzed by a factorial method. We chose correspondence analysis (CA) based on χ^2 metrics[4,28-30] for several reasons: (i) CA confers equal importance to the two fields (rows of molecules and columns of biochemical or biological data) enabling their representation on the same distribution map. The steroid molecules position themselves within this map, which is read with reference to the associated mathematical parameters. (ii) CA groups

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Table I. Relative Binding Affinities (RBAs) for Cytosol Receptors and Relative Tyrosine Aminotransferase (TAT) Activity[a]

	glucocorticoid receptors						progestin receptor: uterus, 24 h	androgen receptor: prostate, 2 h	mineralocorticoid receptor: kidney, 24 h	rel TAT act., %
	HTC		thymus		liver					
	4 h	24 h	4 h	24 h	4 h	24 h				
1, RU 24476	450 ± 80	435 ± 90	228 ± 31	209 ± 24	91 ± 13	99 ± 14	91 ± 12	4.1	1.1 ± 0.8	95
2, desoximetasone	283 ± 22	310 ± 50	208 ± 30	217 ± 28	144 ± 6	163 ± 9	15 ± 4	0.7	84 ± 20	118 ± 8
3, triamcinolone acetonide	190 ± 10	260 ± 40	141 ± 20	174 ± 5	102 ± 6	139	12 ± 1	0.1	8 ± 2	127 ± 10
4, fluocinolone acetonide	180 ± 4	229 ± 22	119	208	129	150 (E)	50 ± 4	0.2	11 ± 2	106 ± 12
5, RU 25253	169 ± 24	163 ± 31	148 ± 21	73 ± 9	50 ± 7	53	530	6.0	2.7	33
6, RU 25055	166 ± 33	80 ± 12	136 ± 26	54 ± 6	118	39	84 ± 16	6 ± 1	2.2	15 ± 5
7, RU 25593	118 ± 19	59 ± 8	66 ± 12	40 ± 9	84 ± 4	54 ± 4	36 ± 3	5 ± 1	0.1	3
8, dexamethasone	100	100	100	100	100	100	≤0.1	≤0.1	21 ± 3	100
9, betamethasone	99 ± 10	80 ± 4	92 ± 5	84 ± 4	92 ± 9	77 ± 11	≤0.1	≤0.1	17 ± 3	106 ± 11
10, 21-desoxydexamethasone	92 ± 4	57 ± 6	67 ± 2	41 ± 5	67 ± 5	49 ± 14	≤0.1	≤0.1	3.1 ± 0.8	45
11, gestrinone	90 ± 5	47 ± 8	96 ± 14	33 ± 6	77 ± 17	18 ± 2	48 ± 8	84 ± 3	1.1	3 ± 2
12, RU 23739	85 ± 11	95 ± 29	140 (E)	40 (E)	8.4	24	67 ± 27	0.3	2.5	110 ± 3
13, corticosterone	85 ± 3	55 ± 4	67 ± 2	22 ± 4	23 ± 6	0.2 ± 0.1	2.8 ± 0.3	0.5 ± 0.2	18 ± 6	71 ± 11
14, 16α-methylprednisolone	82 ± 13	37 ± 8	58 ± 5	33 ± 2	70 ± 1	45 ± 4	≤0.1	≤0.1	2.3	98 ± 9
15, deoxycorticosterone	77 ± 5	40 ± 2	50 ± 7	24 ± 2	0.6 ± 0.1	0.1	18 ± 1	2.4	141 ± 11	17 ± 5
16, 6α,16α-dimethylprogesterone	66 ± 7	42 ± 4	46 ± 3	33	3.9 ± 0.4	1.8 ± 0.2	154 ± 62	8	0.5	26
17, cortivazol (21-OH)	60 ± 10	90 ± 18	150 (E)	117 ± 27	11 ± 5	36	≤0.1	≤0.1	≤0.1	130 ± 15
18, triamcinolone	54 ± 7	38 ± 3	75 ± 3	63 ± 3	84 ± 9	86 ± 18	≤0.1	≤0.1	4.8	81 ± 9
19, RU 2999	42 ± 9	19 ± 4	48 ± 5	12 ± 2	65 ± 10	24 ± 7	303 ± 24	158 ± 14	118 ± 21	8 ± 3
20, hydrocortisone	32 ± 4	15 ± 3	31 ± 4	13 ± 1	41 ± 7	5 ± 2	≤0.1	≤0.1	17 ± 5	66 ± 4
21, 16α-methylprogesterone	30 ± 5	18 ± 3	22 ± 2	12 ± 1	0.5 ± 0.1	0.1	62 ± 10	1 ± 0.3	0.4 ± 0.1	3.5
22, promegestone	24 ± 6	9 ± 3	22 ± 3	6.5 ± 0.7	14 ± 2	3.6 ± 0.8	533 ± 40	1.4 ± 0.4	2.5 ± 0.3	6 ± 3
23, dexamethasone acetate	23 ± 5	30 ± 5	16 ± 3	34 ± 12	43 ± 2	61 ± 4	≤0.1	≤0.1	10	99 ± 10
24, demegestone	17 ± 1	8 ± 2	9 ± 1	2.6 ± 0.3	4.5 ± 0.3	1.2 ± 0.3	420 ± 59	1.1 ± 0.4	0.1	3 ± 3
25, aldosterone	14 ± 2	9 ± 1	14 ± 1	8 ± 1	1.5 ± 0.2	0.1	0.7 ± 0.1	≤0.1	100	49 ± 6
26, cortexolone	12 ± 2	4 ± 1	9.3 ± 0.8	3.3 ± 0.5	≤0.1	≤0.1	0.4 ± 0.1	0.3	28 ± 4	11 ± 4
27, progesterone	12 ± 2	2 ± 1	42 ± 3	17 ± 2	0.2 ± 0.1	≤0.1	100	5.5 ± 0.6	22 ± 6	6 ± 3
28, hydrocortisone acetate	9 ± 3	8 ± 1	3.4 ± 0.3	3.3	10 ± 1	1.3	≤0.1	≤0.1	11 ± 2	91 ± 9
29, RU 22779	7 ± 1	2.5 ± 0.5	10 ± 2	3.0 ± 0.5	1.4 ± 0.5	0.2 ± 0.1	337 ± 99	3.5 ± 1.5	1.8 ± 0.3	3 ± 3
30, RU 18748	1	0.6 ± 0.1	0.7	0.4	≤0.1	≤0.1	≤0.1	≤0.1	2.1 ± 0.8	10 ± 5
31, estradiol	0.8 ± 0.1	0.2 ± 0.1	1.1 ± 0.2	0.3 ± 0.1	0.6 ± 0.1	0.2	0.9 ± 0.1	7.9 ± 1.2	0.5 ± 0.2	6 ± 5
32, testosterone	0.8 ± 0.1	0.3 ± 0.1	0.9 ± 0.3	0.4 ± 0.2	0.2 ± 0.1	≤0.1	1.1 ± 0.3	100	4 ± 1	8 ± 4
33, RU 18760	0.6	0.3 ± 0.1	0.2	0.2	≤0.1	≤0.1	≤0.1	≤0.1	≤0.1	6.5
34, cortisone	0.6	0.4 ± 0.1	0.6	0.2	0.2 ± 0.1	≤0.1	≤0.1	≤0.1	0.8 ± 0.2	9 ± 6
35, norethisterone	ND	5 (E)	ND	1–2	2.8	3 (E)	265	45	0.2 (E)	
36, RU 3097	ND	2 (E)	ND	2 (E)	5.7	1.7	31	19	≤0.1	
37, RU 28289	ND	300 (E)	270	300	ND	173 (E)	440	10*	≤0.1	
38, RU 38140	ND	300 (E)	215	285	ND	170 (E)	85	15*	≤0.1	
39, RU 38486	ND	300 (E)	280	300	106	173 (E)	530	25*	≤0.1	

[a] RBAs were determined as described in the Experimental Section and are expressed as means ±SEM for compounds 1–34. For the factorial analysis, values below 0.1 were arbitrarily set at 0.05. Missing values were estimated (E) on a mathematical basis for compounds 4, 12, and 17 so that they had as little influence as possible on the outcome of the analysis and on the basis of experimental data obtained under slightly different conditions for compounds **35–39**. TAT activity was expressed relative to dexamethasone. ND = not determined; * = value at 24 h.

similar items into classes and breaks each representative class down into its constituent elements (principle of distribution equivalence) without affecting the factors and does not lead to overcontribution to the factorial axes by the elements that carry the most weight. (iii) The descriptive character of CA facilitates the interpretation of available experimental data, as previously shown.[4] (iv) CA offers the possibility of introducing fresh data from ongoing research on new molecules and simulates the positioning of these molecules within the distribution map. (v) CA can estimate the variance of a particular methodology, i.e., appreciate the influence of changes in experimental conditions on the validity of the conclusions drawn. (vi) CA allows the introduction of a further biochemical or biological parameter in order to find out whether it is correlated with the existing system. All these possibilities will be illustrated in the present paper.

Results

Experimental Data. Table I gives the relative binding affinities (RBAs) of the test steroids (Figure 1) for GR, PR, AR, and MR and their relative ability to induce TAT in HTC cells. Competition for GR binding was measured in three cytosols (HTC, thymus, liver) under two sets of incubation conditions (4 and 24 h).

The RBAs of dexamethasone, progesterone, testosterone, and aldosterone for GR, PR, AR, and MR, respectively, were arbitrarily set at 100. None of the steroids apart from the reference molecule, estradiol, competed for binding to the estrogen receptor. The chosen sample voluntarily

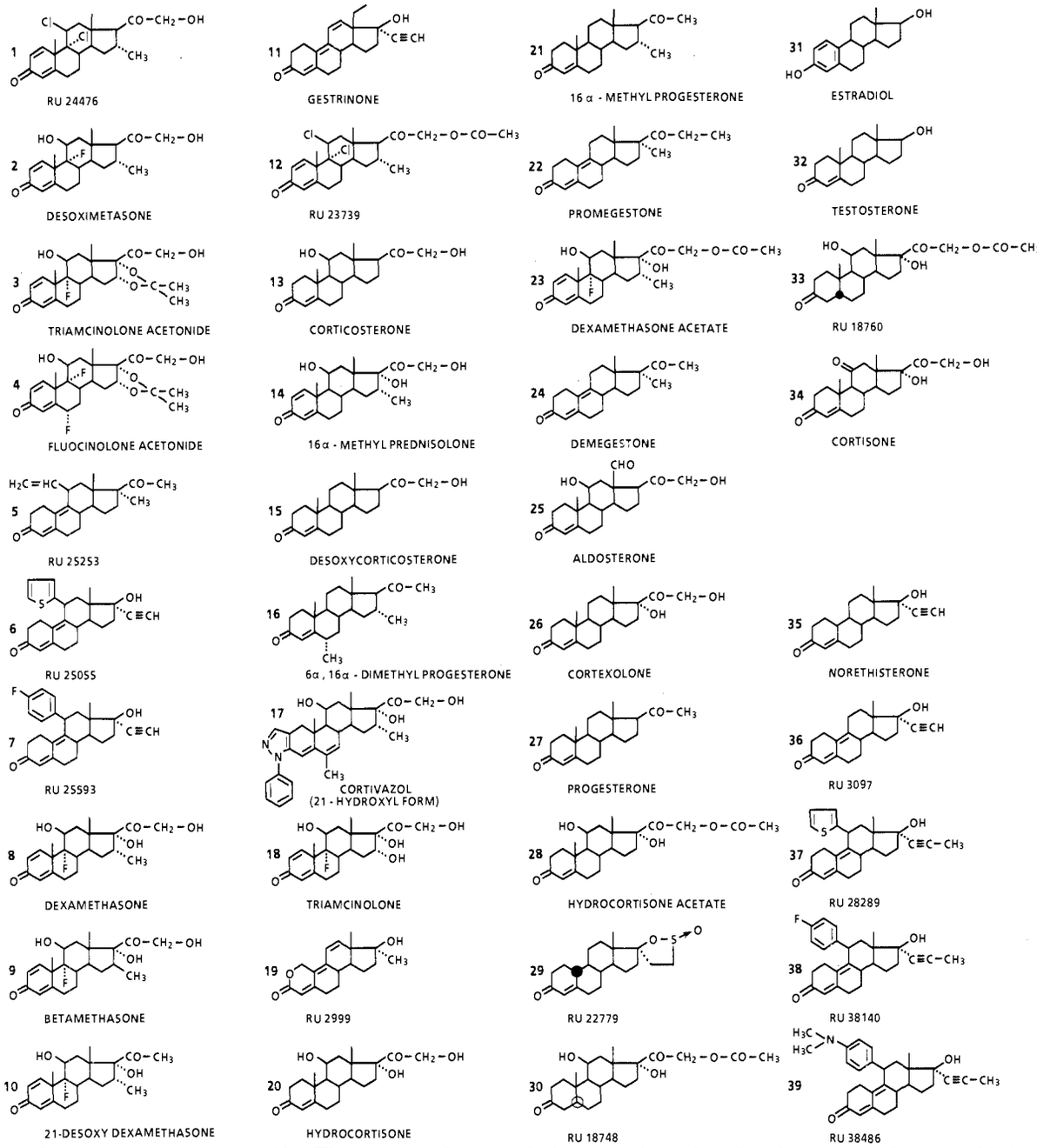

Figure 1. Structures of the test steroids. The correspondence analysis was performed on data for compounds 1–34; additional data on compounds **35–39** were subsequently introduced into the analysis.

reflects a population where progestin and glucocorticoid binding predominate over androgen and mineralocorticoid binding.

Correspondence Analysis. The untransformed data matrix of the RBAs of steroids **1–34** measured at 24 h for GR, PR, and MR and at 2 h for AR was analyzed by CA. This 6D system was thus reduced to several 2D plots of two fields (molecules and receptors). The scatter of the items within each distribution map depicts the relationships among the different receptors and the specificity of binding of the test steroids regardless of the absolute RBA values. Calculation of the five factorial axes ϕ_1–ϕ_5 showed that these account, respectively, for 43.3%, 30.0%, 22.4%, 3.6%, and 0.7% of the total variance of the system.

The population under study is characterized first and foremost by the opposition between GR and PR binding, which contribute 37.9% and 55.6%, respectively, to the ϕ_1 factorial axis, and by the very high similarity in the three GRs (thymus, liver, and HTC cells), which each contribute 11–14% (Table II). The relative contributions (RC) of GR binding to the ϕ_1 factorial axis ($\cos^2 \Theta$) are 0.74 (HTC), 0.86 (thymus), 0.63 (liver); the RC of PR binding is 0.88. The second factorial axis (ϕ_2) reveals the inherent androgenic and mineralocorticoid nature of the molecules (RC = 0.69 and 0.31, respectively) whereas the third axis (ϕ_3) opposes AR and MR binding. Together they amount to 95% of the variance of ϕ_3. The differences among the various GRs, in particular the dichotomy between HTC and liver GR, become apparent when reaching the fourth (ϕ_4) and fifth (ϕ_5) factorial axes.

Four different distribution maps will be considered: ϕ_1 vs ϕ_2 representing 73.3% (43.3% + 30.0%) of the total variance of the system, ϕ_1 vs ϕ_3 representing 65.7% (43.3% + 22.4%) of this variance, ϕ_2 vs ϕ_3 representing 52.4% (30.0% + 22.4%) (not shown), and ϕ_4 vs ϕ_5 representing 4.3% (3.6% + 0.7%) (Figure 2).

A 3D representation of the relative proximity of the different receptors within their field is given by the $\phi_1\phi_2\phi_3$

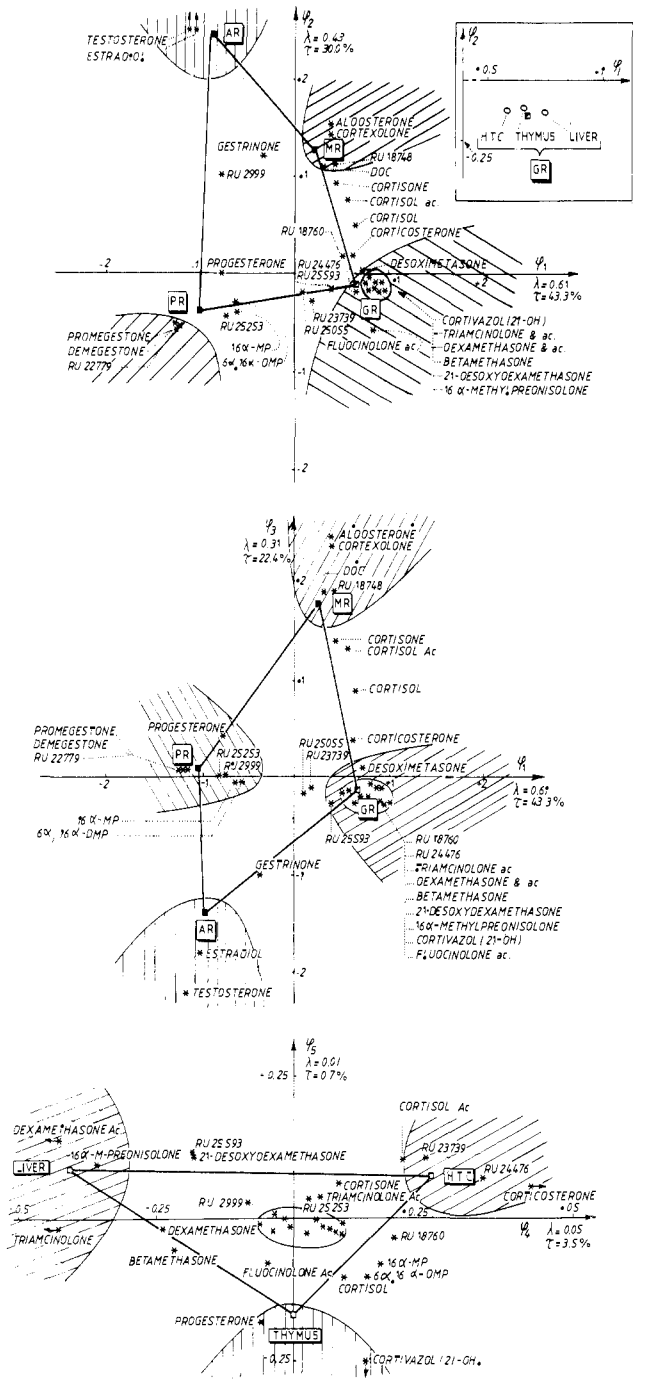

Figure 2. $\phi_1\phi_2$, $\phi_1\phi_3$, and $\phi_4\phi_5$ distribution maps of steroids 1–34 (Figure 1) on the basis of the long-term incubation data in Table I (RBA data at 24 h (GR, PR, MR) or 2 h (AR)). The insert in the top panel shows a blow-up of the GR pole. Each individual GR pole is denoted by an open square (□), the mean GR pole by ◪.

factorial map shown in Figure 3, which embodies 95.7% of the total variance of the system (43.3% + 30.0% + 22.4%).

Relationships between the Receptor and Molecule Fields. Figure 2 (top panel) illustrates clearly that the overall specificity profiles of the molecules under study is not influenced by the choice of system to measure GR binding (thymus, HTC, or liver) since there is virtually a single GR pole (see insert). According to the $\phi_1\phi_2$ distribution map, and the $\phi_1\phi_3$ map, which depicts the opposition between AR and MR binding, the molecules fall into several clusters:

(1) A group of three highly potent pregnane derivatives (**22**, **24**, **29**), two of which are commercialized drugs in France (promegestone, demegestone)[3,31,32] and the third, RU 22779, a sultine analogue.[33] These molecules define the PR pole.

(2) A group of a dozen or so molecules that are fundamentally glucocorticoids characterized by a $COCH_2OH$ function in C17 and a hydroxy group at C11. The majority are well-known drugs such as dexamethasone, betamethasone, 16α-methylprednisolone, fluocinolone acetonide, and desoxydexamethasone (**8**, **9**, **14**, **4**, and **10**, respectively).

(3) A range of seven molecules between these two poles: (a) Progesterone (**27**), 16α-methylprogesterone (**21**),

Table II. Absolute and Relative Contributions of the Biochemical Parameters to the Factorial Axes

	ABSOLUTE CONTRIBUTIONS		RELATIVE CONTRIBUTIONS COS^2
ϕ_1			
PR	55.6%		0.8791
GR-HTC	13.77%		0.7374
GR-Thymus	12.47%		0.8596
GR-Liver	11.66%		0.6348
AR	5.89%		0.0927
MR	0.58%		0.0111
ϕ_2			
AR	63.93%		0.6969
MR	23.54%		0.3113
PR	10.57%		0.1158
GR-HTC	0.91%		0.0339
GR-Liver	0.52%		0.0199
GR-Thymus	0.5%		0.0239
ϕ_3			
MR			0.6771
AR	25.92%	68.8%	0.2103
GR-Liver	2.41%		0.0679
GR-HTC	1.2%		0.0331
GR-Thymus	1.08%		0.0385
PR	0.57%		0.0046
ϕ_4			
GR-Liver	59.06%		0.2653
GR-HTC	40.45%		0.1787
PR	0.23%		0.0003
MR	0.22%		0.0003
AR	0.02%		0.0000
GR-Thymus	0%		0.0000
ϕ_5			
GR-Thymus			0.0778
GR-HTC	18.71%	68.03%	0.0166
GR-Liver	13.2%		0.0119
MR	0.04%		0.0000
PR	0%		0.0000
AR	0%		0.0000

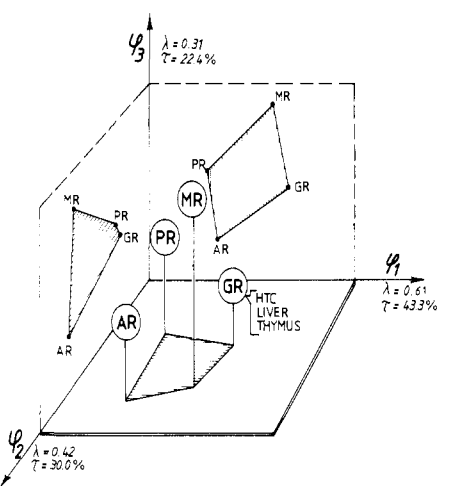

Figure 3. Positioning of the receptors within the $\phi_1\phi_2\phi_3$ distribution map. The projections $\phi_1\phi_2$, $\phi_1\phi_3$, $\phi_2\phi_3$ are shown but not the positions of the test steroids.

(31) Raynaud, J. P.; Ojasoo, T. *J. Gynecol. Obstet. Biol. Reprod.* **1983**, *12*, 697.
(32) Raynaud, J. P.; Ojasoo, T.; Pottier, J.; Salmon, J. In *Biochemical Actions of Hormones*; Litwack, G., Ed.; Academic: New York, 1982; Vol. IX, p 305.
(33) Nédélec, L.; Torelli, V.; Rousseau, G.; Allais, A.; Philibert, D.; Fournex, R.; Azadian-Boulanger, G. *J. Steroid Biochem.* **1978**, *9*, 814.

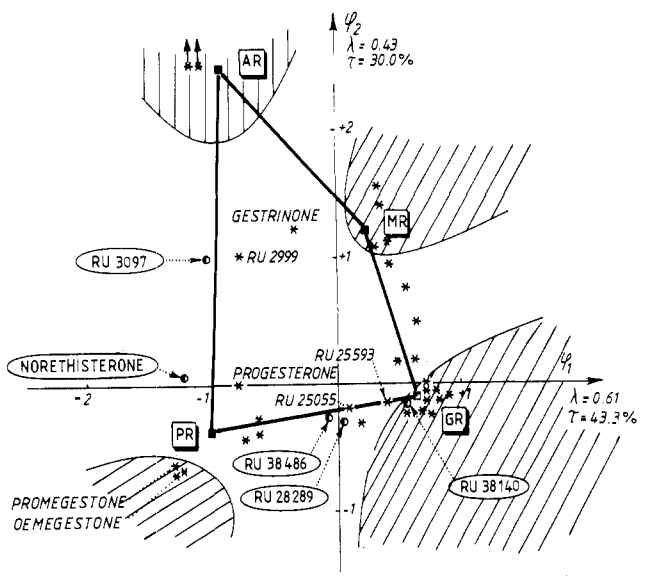

Figure 4. Location within the $\phi_1\phi_2$ map shown in Figure 2 of the additional test molecules **35–39**.

6α,16α-dimethylprogesterone (**16**), and the 11β-vinyl derivative of demegestone, RU 25253 (**5**), which is a more potent progestin than demegestone itself,[5,34] are all pregnane derivatives and remain decidedly within the influence of the PR pole. However, of these molecules, only progesterone is subject to a pull from AR and MR similar to that from GR reflecting its known androgenic, mineralocorticoid, and glucocorticoid antagonism. (b) RU 25055 (**6**) and RU 25593 (**7**), which are norethisterone derivatives with a C9(10) double bond and a *p*-fluorophenyl or thienyl substituent at C11,[35] and RU 23739 (**12**), the acetate of RU 24476 (**1**), are, on the other hand, in the zone of the GR pole in spite of the general preconception that norethisterone derivatives are androgenic progestins. RU 25055 is inactive as a glucocorticoid but is a potent antagonist of dexamethasone in vitro. In the case of RU 23739, the presence of the acetate group may explain its more progestin character.

To clarify the positions of RU 25055 and of RU 25593, the location of the parent compound, norethisterone (**35**), absent from the initial table, was estimated from the additional data in Table I and found to be situated, as expected, well within the PR zone but subject to a pull from the AR pole (Figure 4). The location of the unsaturated Δ^9 derivative (RU 3097 (**36**)) of norethisterone was even further toward AR. It is thus evident that the C11 substituent in RU 25055 and RU 25593 introduces or reinforces an existing GR binding component. The positions of the 17α-propynyl analogues of these compounds, RU 28289 (**37**) and RU 38140 (**38**), respectively (Figure 1 and Table I), were adjacent to the ethynyl parent compounds, but the shift in opposite directions remains unexplained. The 11-[(dimethylamino)phenyl]-17α-propynyl derivative, RU 38486 (**39**),[35] represents the most beautiful compromise between PR and GR binding.

The above comments are valid for the population under study, but their absolute validity is subject to caution. Tritiated promegestone and dexamethasone were used to label PR and GR in our screening system although they do not exhibit total specificity toward their respective receptors. More specific glucocorticoids are now available (RU 26988, RU 28362: 11β,17β-dihydroxy-17α-(1-propynyl)androsta-1,4,6-trien-3-one and its 6-methyl derivative, respectively)[3,36,37] but, to our knowledge, a progestin that does not interfere with GR has not yet been identified. Furthermore, it should be borne in mind that PR have been identified in the thymus and HTC cells[38-40] and that GR is present in the uterus.[41,42]

(4) Mineralocorticoid binding is represented by aldosterone, 11-deoxycorticosterone, cortexolone, principally known for its antiglucocorticoid activity, and RU 18748. RU 18748 is an ineffective overall competitor possibly because of the 5α-configuration and the need to be deacetylated for binding activity.

(5) A range of compounds is scattered along the mineraloglucocorticoid junction suggesting a gradation between these two binding aspects. This observation could be explained by the presence of both MR and GR binding sites in the kidney and liver cytosols to which the radioligands aldosterone and dexamethasone bind to different extents and at varying rates and which are not similarly competed for by test steroids with different specificities and interaction kinetics for these sites. Dexamethasone can bind to MR,[1,2,43,44] and it has been estimated that in kidney slices at 37 °C it has almost 30% of the affinity for MR that it has for GR. The mineralocorticoid to glucocorticoid affinity ratio of a series of glucocorticoids was found to be ranked as follows: DOC > 9α-fluorocortisol > cortisol = corticosterone > prednisolone = betamethasone = dexamethasone.[45] This ranking is the same as that found here. Thus, in the absence of a binder, specific to one type of site such as RU 26988 or RU 28362 mentioned above, now systematically used in our screening system, heterogeneous binding is measured.

(6) The androgen pole is poorly represented just by testosterone (**32**) but also by estradiol (**31**). It is known that this is the most important binding component of estradiol when its binding to the estrogen receptor is disregarded (Table I).

(7) Gestrinone (**11**) and RU 2999 (**19**), as has already been observed on several occasions, lack specificity on account of the triene ($\Delta^{4,9,11}$) structure, which distinguishes them from the other test molecules and which results in a flat highly flexible molecule presumably able to fit into all the steroid receptor binding sites with relative ease.[46] Because they do not have an electron donor in C3, however, they apparently lack the ability to interact with ER.

It is the $\phi_4\phi_5$ distribution map (bottom panel of Figure 2) that differentiates the three GRs (HTC, thymus, liver)

(34) Teutsch, G.; Bélanger, A.; Philibert, D.; Tournemine, C. *Steroids* **1982**, *39*, 607.
(35) Teutsch, G. In *Adrenal Steroid Antagonism*; Agarwal, M. K., Ed.; Walter de Gruyter: Berlin, 1984; p 43.
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(44) Funder, J. W.; Feldman, D.; Edelman, I. S. *Endocrinology* **1973**, *92*, 1005.
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(46) Delettrē, J.; Mornon, J. P.; Lepicard, G.; Ojasoo, T.; Raynaud, J. P. *J. Steroid Biochem.* **1980**, *13*, 45.

and reveals the relative positioning of the test molecules with respect to these receptors. In the absence of large experimental errors, the scatter observed could reflect three phenomena: differential metabolic stabilities of the ligands in these cytosols even at 0 °C, different specificities of GR according to tissue, and different proportions of GR and MR binding sites in these systems to which the ligands bind to dissimilar extents. It has been reported that whereas the enzyme systems may remain operational in normal liver cytosols, their activity is considerably diminished in cytosols from transformed cells (e.g., hepatoma cells).[47-49] Hydrocortisone (**20**), desoxycorticosterone (**15**), and cortexolone (**26**) are not metabolized in HTC cells whereas dexamethasone is metabolized to about 10–15% and progesterone completely. RU 38486 (**39**) was not metabolized in HTC cells after 3-h incubation but was extremely metabolized in cultured liver cells. A few studies refer to differences in GR specificities according to tissue and species but more definite data will be available when the cDNA of GR from different origins have been cloned and sequenced.[19-21,50] The different specificity of rat liver GR, which contrasts with the similar specificity of rat heart, pancreas, and kidney GR, would be due to the presence of a heat-labile cytosol factor.[51] On the other hand, human spleen tumors and rat livers have apparently identical GR binding sites.[52] In a given species, GR seem to bind better those steroids that are normally secreted by that species than steroids that are not secreted.[53] In the bottom panel of Figure 2, a central cluster of a dozen molecules gives the same results regardless of the choice of system. Those closest to the liver, the transforming organ par excellence, might be the more resistant to metabolic degradation, i.e., triamcinolone (**18**), dexamethasone acetate (**23**), 16α-methylprednisolone (**14**). Those furthest away are probably more susceptible to inactivation by the liver than by the other tissues, i.e., 16α-methylprogesterone (**21**), 6α,16α-dimethylprogesterone (**16**), hydrocortisone (**20**). Differences have already been observed in the competitive ability of progesterone and 16α-methylprogesterone versus that of thymus and liver GR. Competition in liver, but not in thymus cytosol, was markedly affected by the concentration of protein and the isotonicity of the homogenate.[54]

It is possible to assess the stability of the complex formed between ligand and receptor, by comparing RBAs under short- and long-term incubation conditions. A relative increase in RBA with respect to the natural hormone on prolonging incubation time implies that the test compound forms a more slowly dissociating complex with the receptor than does the natural hormone.[55] A decrease in RBA can indicate the formation of a fast-dissociating complex but also the conversion of the test substance to low-affinity degradation products. We have previously analyzed the influence of incubation time on RBAs for GR in these three tissues and shown by classic two-by-two

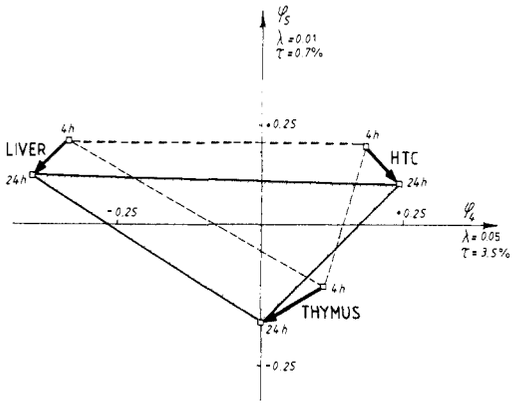

Figure 5. Displacement of GR poles within the $\phi_4\phi_5$ distribution map as a function of incubation time.

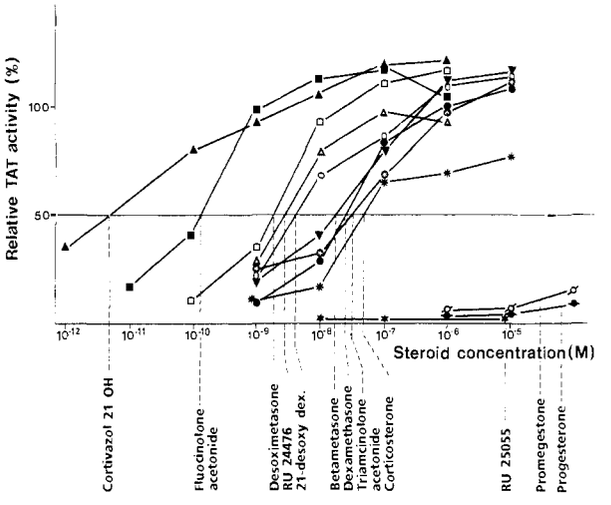

Figure 6. TAT activity as a function of glucocorticoid concentration.

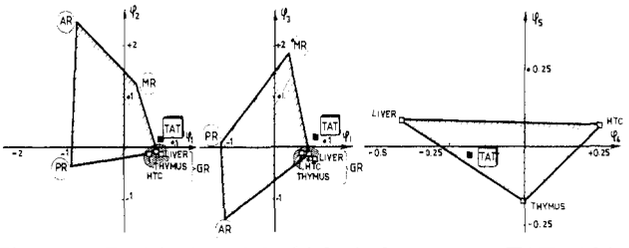

Figure 7. Positioning of the biological parameter TAT within the panels of Figure 2.

correlations that there are excellent correlations (r = 0.97) between RBAs in cytosols from HTC cells and thymus after 4- or 24-h incubation at 0 °C, suggesting either that there is little degradation under these conditions or that any degradation that may occur is strikingly similar in both systems.[6] On the other hand, correlations between thymus and liver values were less good (r = 0.70), suggesting that many of the test substances might undergo extensive degradation by the liver and/or interact with more than one binding protein. Figure 5 presents the same information analyzed by CA and depicts the variance of the GR measuring system. The increase in incubation time from 4 to 24 h has accentuated the dispersion of the poles, but the displacement of the GR pole for the liver is no greater than for the two other systems, suggesting that metabolic effects are not the only explanation for the variations observed.

Introduction of a Biological Parameter. TAT Induction. On inclusion of the data on TAT induction in HTC cells (last column of Table I and Figure 6) into the

(47) Samuels, H. H.; Tomkins, G. M. *J. Mol. Biol.* **1970**, *52*, 57.
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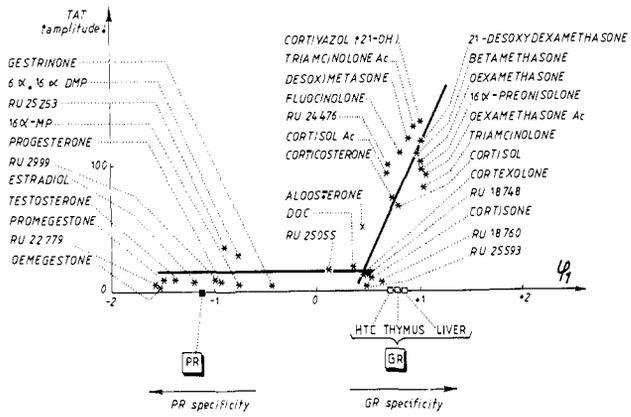

Figure 8. TAT amplitude as a function of PR–GR specificity.

analysis, it was observed that this biological parameter was highly correlated with binding to GR and not to any of the other receptors (Figure 7). It is therefore a true expression of glucocorticoid activity.

In order to establish whether there was any correlation between the amplitude of this in vitro biological response and glucocorticoid specificity, we plotted amplitude against the projection coordinates on the ϕ_1 factorial axis (Figure 8) and observed an excellent correlation for well-known glucocorticoids in confirmation of earlier results by many teams.[56-58] The most potent compound was the much-studied derivative cortivazol;[59,60] the least active compounds in this series were derivatives without a hydroxyl function in the 11β-position or with a C11 keto group, i.e., cortexolone (**26**), an accredited antiglucocorticoid,[61-63] desoxycorticosterone (**15**), and cortisone and Δ^4-reduced compounds, i.e., RU 18748 (**30**) and RU 18760 (**33**). More interesting was the observation that, with the exception of the two C11 substituted derivatives RU 25055 (**6**) and RU 25253 (**5**) and of 6α,6α-dimethylprogesterone (**16**) that exhibited some glucocorticoid potential, the remaining compounds, virtually all progestins, showed no glucocorticoid agonist action in this test regardless of their relative glucocorticoid specificity. We have previously reported that progestins have antiglucocorticoid action in several test systems[5,6] but with the implicit assumption that some might have partial agonist activity under certain circumstances as indeed reported by others.[64] The present analysis however would appear to support the more recent contention that the activity of the antiglucocorticoid–GR complex is zero on TAT expression in HTC cells and that they are antagonists by virtue of their binding to the natural hormone binding site, which they usurp but do not activate in vivo.[65] In this way the number of functional

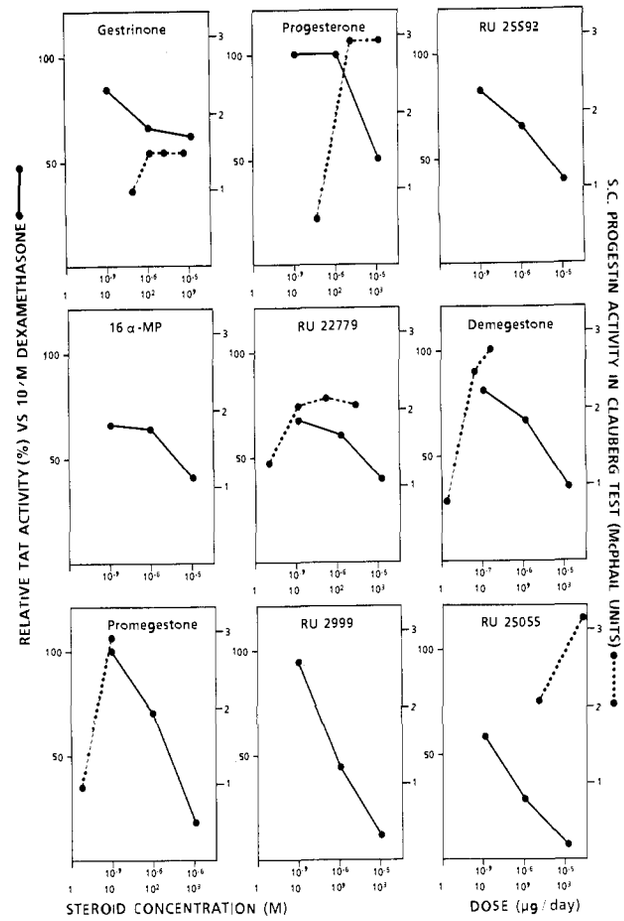

Figure 9. Progestomimetic (ascending curves) and antiglucocorticoid (descending curves) activities of nine test compounds.

GR receptors is reduced and activity is decreased. Surprisingly, however, the antagonist activity of some of these compounds on dexamethasone-induced TAT was highly similar (Figure 9). Binding studies on whole cell assays might differentiate the compounds better. The magnitude of several transcriptional responses elicited by the receptor are roughly proportional to the number of receptor molecules per cell.[66] Further studies could also include the measurement of other biological responses in the same or different cell systems in order to establish, via the analysis of large series of compounds, how these responses relate to each other. It has already been demonstrated that independently derived hepatoma cell lines (HTC and Fu5-5) can display different sensitivities for the induction of TAT[67-69] and that differential in vivo antagonism of glucocorticoid responses can coexist in the same model system.[70]

Discussion

To interpret with confidence vast numbers of RBA measurements influenced by uncontrolled factors such as different receptor concentrations, different radioligand specificities, in vitro metabolism, ..., a powerful multiparametric methodology is required. The present ap-

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(70) Naylor, P. H.; Gilani, S. S.; Milholland, R. J.; Ip, M.; Rosen, F. *J. Steroid Biochem.* **1981**, *14*, 1303.

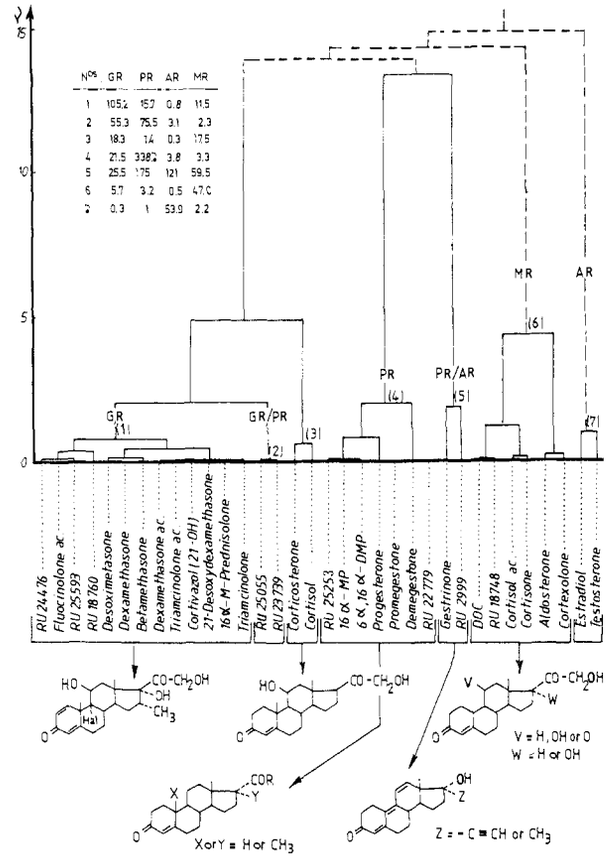

Figure 10. Hierarchical ascending classification of steroids 1–34 based on the projection coordinates on axes $\phi_1\phi_2\phi_3$.

proach using correspondence analysis eliminates such background interference and consequently guards against artefactual interpretation. The metabolic variations that may influence results have been shown to be less important than affinity differences since they only become apparent on lower factorial axes. The analysis has highlighted the distinctive nature of each receptor class (since the three GRs of different sources are only distinguished at the level of the $\phi_4\phi_5$ distribution map) and has also revealed the mixed specificity of chemically related ligands and thus the degree of similarity that may exist among these receptor proteins.

The power of the methodology has been illustrated by the introduction of data on new test compounds into the original matrix and also of results on a further (biological) parameter. Topical results can thus be related to existing data. The antiglucocorticoid activity of PR binders has been confirmed in this way. Indeed the initial selection of molecules was not governed by a true structure–affinity search but by the intuition that many progestins could interfere with glucocorticoid binding and have antiglucocorticoid activity. To show how the molecules can relate to each other structurally, we have used a complementary method derived from the factorial analysis. The 34 steroids have been classified into five archetypes on the basis of their binding to the four receptors by using the projection coordinates on the first three factors to filter essential information and by using an algorithm for a hierarchical ascending classification based on a two-center moment as an aggregation criterion (Figure 10). A similar approach has been applied to all biochemical and biological response parameters (Figure 11).

With the availability of in vitro expression systems with cloned receptor proteins for the study of these molecules, further data can be injected into the present data matrix

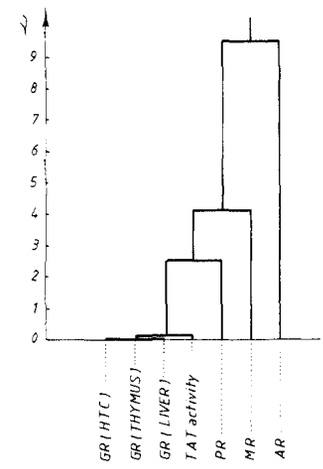

Figure 11. Hierarchical ascending classification of biochemical and biological response parameters.

in order to confirm and refine conclusions already reached with cruder tests.

Experimental Section

Correspondence Analysis. Calculations were performed on a microcomputer (16–32 bits of 655 kilobytes of central memory, Hewlett-Packard 9836) with a CA program transcribed into BASIC from a FORTRAN Anacor software. The factorial maps were drawn directly on a digital plotter with a precision of $^1/_{100}$ in. (but have been redrawn by a professional artist for the purposes of this paper).

Relative Binding Affinity Determinations. Each test steroid is incubated with preparations containing supposedly high concentrations of the receptor under study ("cytosol") and a radioactively labeled marker known to bind as selectively as possible to this receptor. Bound radioactivity is separated by a dextran-coated charcoal (DCC) adsorption method. The percent radioligand bound in the presence of test steroid compared to that bound in its absence is plotted against the concentration of competing test steroid. A standard curve for the competition of unlabeled radioligand is constructed with the use of 9–10 concentrations; five to six concentrations of each test steroid are used. From this plot, the molar concentrations of unlabeled radioligand or steroid competitor that reduce radioligand binding by 50% are determined. The effectiveness of the competitor is given by the ratio of the concentrations of unlabeled radioligand and of test steroid for 50% competition. This ratio multiplied by 100 is the relative binding affinity or RBA. In this study, RBAs were determined in at least three different experiments, and the mean RBA was calculated.

To prepare cytosols for the determination of binding to GR, thymus and perfused livers from adrenalectomized rats were homogenized (1/10, wt/vol) in 10 mM Tris·HCl buffer (pH 7.4) containing 0.25 M sucrose (livers) and 2 mM dithiothreitol (DTT) (thymus). HTC cells in the log phase of growth ($\sim 5 \times 10^5$ cells/mL) were sedimented at 600g for 10 min, washed twice with the DTT-containing buffer, frozen, and stored at −20 °C for less than 15 days. Upon use, they were thawed and then homogenized in this buffer. Cytosols were prepared by centrifuging homogenates at 105000g for 60 min at 4 °C and incubated with 5 nM [^{3}H]dexamethasone at 0 °C for 4 or 24 h in the presence of 0–2500 nM unlabeled competing steroid.

Cytosols for PR, AR, and MR competition were obtained by homogenization of the following organs in Tris–sucrose buffer and subsequent centrifugation: uteri (1/50, wt/vol) from estradiol-primed immature rabbits (PR), prostates (1/5, wt/vol) from castrated rats (AR), and kidneys (1/3, wt/vol) from rats (MR). These cytosols were incubated with 2.5 nM [^{3}H]promegestone, 2.5 nM [^{3}H]methyltrienolone and 2.5 nM [^{3}H]aldosterone, respectively, in the presence of 0–2500 nM unlabeled competing steroid.

Determination of Tyrosine Aminotransferase (TAT) Activity.[58] HTC cell suspensions were grown at 37 °C in modified SWIM'S S_{77} medium containing 10% calf serum. The medium was buffered with 0.5 g of $NaHCO_3$/L and 5×10^{-2} M tricine

adjusted to pH 7.4 at 37 °C. In this medium, the cells have a generation time of 24–30 h and remain in the log phase of growth between a cell density of (2–8) × 10^5 cells/mL. All incubations were carried out in spinner flasks at 37 °C under magnetic stirring (100 rpm). Test steroids were added in a 10 μL solution of ethanol per 10 mL of culture (density 2 × 10^5 cells/mL). Equivalent amounts of ethanol were added to control samples. Cell viability was tested by trypan blue exclusion. To assay TAT, 2-mL samples of the cell culture were removed, and the cells were collected by centrifugation. The pellets were washed twice in isotonic saline and thereafter suspended and chilled in 1 mL of 5 × 10^{-2} M potassium phosphate buffer (pH 7.6), 2 × 10^{-3} M 2-oxoglutarate, 1 × 10^{-4} M pyridoxal phosphate, and 1 × 10^{-3} M EDTA. The cells were disrupted with an ultrasonicator (250 TS-20K, Annemasse, France) by two consecutive exposures to 70 V for 20-s bursts. The enzyme was assayed at 37 °C by the method of Diamondstone.[71] One unit of activity represents the formation of 1 μmol of *p*-hydroxyphenylpyruvate/minute. Enzyme-specific activity is expressed as milliunits of TAT/milligram of cell protein. The protein content was measured by the method of Lowry et al. with BSA as standard. The maximum steady-state enzymic activity obtained with 10^{-6} M dexamethasone (70–80 milliunits/mg of protein) corresponds to 100% in Table I.

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Registry No. 1, 65049-45-8; 2, 382-67-2; 3, 76-25-5; 4, 67-73-2; 5, 67983-60-2; 6, 67983-56-6; 7, 67983-73-7; 8, 50-02-2; 9, 378-44-9; 10, 426-15-3; 11, 16320-04-0; 12, 65049-43-6; 13, 50-22-6; 14, 10417-63-7; 15, 64-85-7; 16, 1816-78-0; 17, 4906-84-7; 18, 124-94-7; 19, 124-94-7; 20, 50-23-7; 21, 1239-79-8; 22, 34184-77-5; 23, 1177-87-3; 24, 10116-22-0; 25, 52-39-1; 26, 152-58-9; 27, 57-83-0; 28, 50-03-3; 29, 74183-64-5; 30, 4004-68-6; 31, 50-28-2; 32, 58-22-0; 33, 64313-94-6; 34, 53-06-5; 35, 68-22-4; 36, 14531-92-1; 37, 88256-64-8; 38, 88256-65-9; 39, 84371-65-3; TAT, 9014-55-5.

Supplementary Material Available: Details of correspondence analysis, i.e., coordinates and relative and absolute contributions (3 pages). Ordering information is given on any current masthead page.

Tricyclic Compounds as Selective Antimuscarinics. 2. Structure–Activity Relationships of M_1-Selective Antimuscarinics Related to Pirenzepine

Wolfgang G. Eberlein,*† Wolfhard W. Engel,† Günter Trummlitz,† Günther Schmidt,†,‖ and Rudolf Hammer‡,§

Department of Chemical Research, Dr. Karl Thomae GmbH, D-7950 Biberach 1, Germany, and Department of Biochemistry, Istituto de Angeli S.p.A., Milano, Italy. *Received July 2, 1987*

In order to gain some insight into those structural features that control M_1 selectivity, a selected set of pirenzepine analogues has been studied in which both the tricyclic ring system and the basic side chain have been varied. Binding studies were conducted in rat tissue homogenates from cerebral cortex (M_1) and gastric fundus (M_2). The ratio of IC_{50} values of the test compounds in the two different tissues was taken as a measure of M_1 receptor selectivity. Several derivatives, especially those with flexible side chains, i.e. high degree of freedom of rotation around single bonds, proved to be nonselective. Among semirigid compounds only those containing 6-membered ring systems (11, 13, 14, and 15) showed significant M_1 selectivity. Principles of structure–activity and structure–selectivity are discussed.

Pirenzepine (2) is a tricyclic drug that unlike psychotropic tricyclic agents exhibits measurable inhibitory effects exclusively toward the muscarinic receptor system.[1] The underlying structural requirements for selectivity toward the muscarinic acetylcholine receptor have been discussed in a preceding paper.[2] Pirenzepine (2) has been introduced into ulcer therapy, providing safe and unproblematic treatment of gastritis and duodenal ulcer.[3] Both experimental and clinical evidence point to the fact that the therapeutic effects of pirenzepine are due to a selective blockade of M_1 receptors governing gastric secretion.[4] In humans, significant reduction of gastric acid and pepsinogen secretion is obtained at plasma levels at which other antimuscarinic effects, like mydriasis, inhibition of gastric emptying, inhibition of salivation, and impairment of esophageal motility, do not occur. Moreover, tachycardia, a common side effect of classical antimuscarinics, is not observed following pirenzepine treatment.[5]

Receptor-binding studies have provided the first insight into the mode of action of pirenzepine (2). It has been shown that this compound is able to discriminate between high- and low-affinity binding sites within the muscarinic acetylcholine receptor system. Outside the central nervous system, the high-affinity subtype appears to be prevalent in the sympathetic and myenteric ganglia whereas low-affinity subtypes are found in high proportions in peripheral muscarinic effector organs such as heart, exocrine glands, and smooth muscle.[6] An evolving classification scheme for these muscarinic receptors divides them into M_1 (high affinity) and M_2 (low affinity) subtypes. Although details of the distinctions are still in the beginning, this characterization offers a sound basis on which to compare the binding properties of different muscarinic antagonists and to assess their potential M_1 selectivity.

As part of our interest in the development of agents that selectively interact with the muscarinic system, we have

† Dr. Karl Thomae GmbH.
‡ Istituto de Angeli S.p.A.
§ Present address: Biochemistry, Boehringer Ingelheim Zentrale GmbH, D-6507 Ingelheim, Germany.
‖ Deceased.

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