MTD Approach to Quantitative Structure-Activity Relationships for Cardiotonic Steroids

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A minimal topological difference (MTD) approach is made to describe quantitative structure-activity relationships (QSAR) for the Na^+, K^+-ATPase inhibitory activity of cardiotonic steroids. The calculations take into account 20 derivatives of digitoxigenin, digoxigenin, and gitoxigenin with small substituents at different sites of the steroid backbone. A multiple correlation coefficient of $r = 0.916$ is obtained using the MTD and an indicator variable for the presence of a 15β substituent. The corresponding receptor map reveals receptor wall vertices in the C11, C12, C15, and C22 regions. Both 3β and 16β substituents are found to contain receptor cavity vertices. The MTD results are discussed with respect to lactone-ring conformational investigations presented and they are compared with findings of previous structure-activity studies.

Introduction

Receptor site mapping by the only use of structure and activity data of various steroid molecules was performed on the basis of X-ray crystal structure information [1–5] or potential-energy calculations [6, 7]. However, these techniques were mainly applied to locate possible hydrogen bonding zones. For a more general approach to receptor characterization, the Minimal Topological Difference (MTD) method [8, 9] was developed. An application of this method to cardiotonic steroids by use of toxicity data is given in [10, 11]. But there are some indications that the toxic effect can be separated from the inotropic one as a precondition for the development of new cardiotonic steroids which have an improved safety index [12–14]. The membrane-bound Na^+, K^+-ATPase or a part of this enzyme is assumed by some investigators [15–17] to be the cardioactivity receptor or a proper model for it.

In the present paper, guinea-pig heart Na^+, K^+-ATPase inhibition data of 20 cardiotonic steroids are investigated by means of the MTD method. Only relatively small substituents are considered in order to avoid vertex number expansion in the MTD scheme. Steroid-backbone double bonds and other chemical modifications which are supposed to produce drastic structural changes and, therefore, difficulties in the MTD superposition process will not be included into the calculations. Potential-energy calculations on lactone-ring orientations of selected steroids are performed to complement the discussion of MTD results.

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Materials and Methods

The $n = 20$ steroids employed in the MTD study are presented in Table I together with the inverse logarithms of molar concentration for half-maximum inhibition of Na^+, K^+-ATPase activity ($-\log H_{50}$) experimentally determined under comparable conditions [18–23]. Fig. 1 illustrates the hypermolecule indicating the topological network of all the superimposed non-hydrogen atoms which occur in the compounds under study. The steric structure of a particular molecule i is described in the structure matrix x which contains elements for each of the $m = 16$ vertices j of this hypermolecule (with $x_{ij} = 1$ in case of occupation and $x_{ij} = 0$ otherwise). Steroid-backbone and lactone-ring vertices which are common to all molecules are not numerotated. All numbered vertices are attributed to receptor cavity ($\varepsilon_j = -1$), receptor wall ($\varepsilon_j = +1$) or sterically irrelevant vertices ($\varepsilon_j = 0$) by a trial and error procedure [9]. The optimized receptor map S* corresponds to the vector ε

for which the best fit between experimental activities and calculated MTD data is achieved. The MTD value for the molecule i is given by:

$$\mathrm{MTD}_i = s + \sum_{j=1}^{m} \varepsilon_j x_{ij} \qquad (1)$$

where s is the total number of cavity vertices. In addition to the MTD parameter, an indicator variable δ_7 is introduced due to the strong inhibitory effect of C15 substituents (vertex $j = 7$) [17].

Investigations on lactone-ring conformations of digitoxigenin derivatives were performed by use of the program [24] on an EC 1040 computer. The potential energy is obtained by the sum of non-bonded interactions between all atoms k ≠ l of the molecule:

$$U_{nb} = \sum_{k,l} [a_{kl} \exp(-b_{kl} r_{kl})/(r_{kl}{}^{d_{kl}}) - c_{kl}/r_{kl}{}^{6}] \qquad (2)$$

where r_{kl} is the distance between atom k and l. The *a, b, c, d* parameters are taken from [25]. In the calculations of the potential-energy curves for lactone-ring rotation about the C17–C20 bond, the orientations of methyl groups at C13, C21, and C22 are optimized for each point of these curves. Bond lengths and valence angles are taken from the crystallographic investigation of digitoxigenin [26] and held fixed during the optimizations. The numerotation of steroid-skeleton carbon atoms can be seen in Fig. 1b.

Results

Strongly subdivided receptor-cavity spheres are difficult to reconcile with chemical intuition at first glance. Therefore, cavity vertices should form a single-connected network. Following this rule the receptor map S_1^* and the corresponding regression equation (3) were determined:

$$S_1^* \begin{cases} j(\varepsilon = -1)\text{: } 1, 2, 16 \\ j(\varepsilon = 0)\text{: } 3, 8-12, 15 \\ j(\varepsilon = +1)\text{: } 4-7, 13, 14 \end{cases}$$

$$A_1{}^{\mathrm{theor}} = 6.401 - 0.669\,\mathrm{MTD} - 0.863\,\delta_7 \qquad (3)$$
$$n = 20 \quad r = 0.858 \quad s = 0.434 \quad F_{2,17} = 23.8$$
$$\alpha < 0.001$$

A significant improvement in the multiple correlation coefficient can be obtained by use of the receptor map S_2^* in deviation from the single-connected network rule:

$$S_2^* \begin{cases} j(\varepsilon = -1)\text{: } 1, 2, 9, 16 \\ j(\varepsilon = 0)\text{: } 3, 10-12, 15 \\ j(\varepsilon = +1)\text{: } 4-8, 13, 14 \end{cases}$$

$$A_2{}^{\mathrm{theor}} = 7.383 - 0.753\,\mathrm{MTD} - 0.923\,\delta_7 \qquad (4)$$
$$n = 20 \quad r = 0.916 \quad s = 0.340 \quad F_{2,17} = 44.2$$
$$\alpha < 0.001$$

An illustration of receptor map S_2^* is presented in Fig. 1a. Theoretical activity values A^{theor} calculated with respect to both receptor maps for all the steroids under consideration can be taken from Table I.

The calculated potential-energy curves for 17β side-chain rotation of C21 or/and C22 substituted digitoxigenin derivatives are given in Figs. 2 and 3. Both the (21S) and the (21R) methyldigitoxigenin are found to preferably adopt the (C22, O14) conformation with broad potential wells about +40° to +80° in the torsional angle C13–C17–C20–C22. Compared with the other potential minima due to (C21, O14) conformations, the energy differences are calculated to be 123.3 and 42.9 kJ mol^{-1} for both steroids, respectively. On the contrary, the

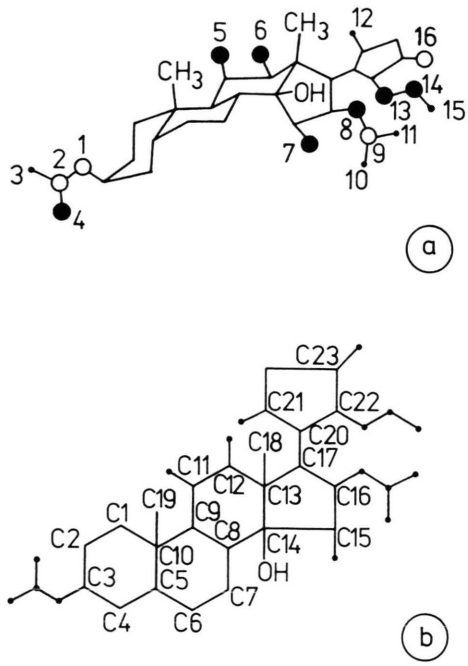

Fig. 1. Schematic representations of the hypermolecule for MTD calculations a) with vertex numerotation (for receptor map S_2^*: ○ receptor cavity vertex; ● receptor wall vertex; • sterically irrelevant vertex) and b) with numerotation of steroid-skeleton carbon atoms.

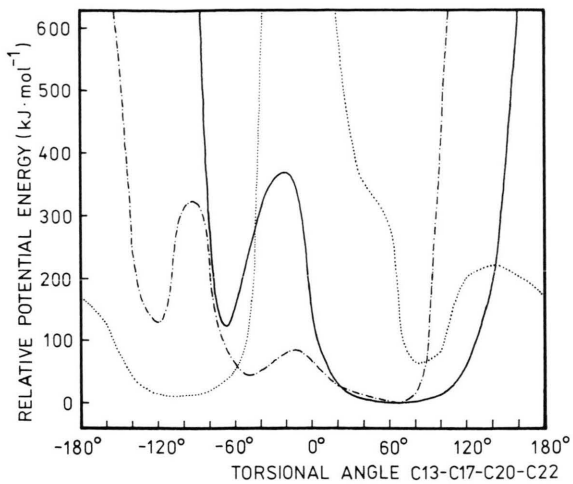

Fig. 2. Potential-energy curves for lactone-ring rotation of monosubstituted digitoxigenin derivatives:
——— (21S)-21-methyldigitoxigenin;
–·–·– (21R)-21-methyldigitoxigenin;
..... 22-methyldigitoxigenin.

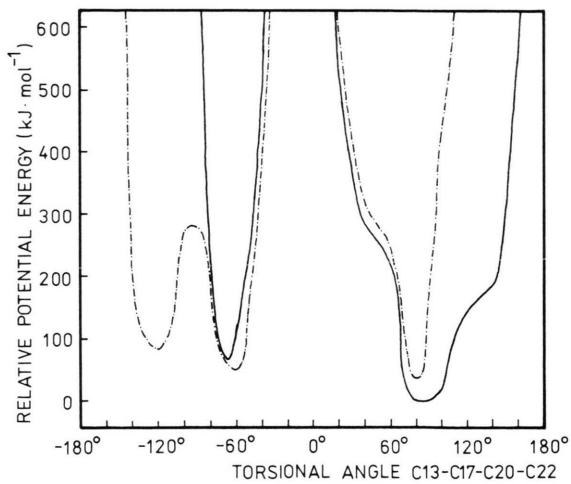

Fig. 3. Potential-energy curves for lactone-ring rotation of disubstituted digitoxigenin derivatives:
——— (21S)-21,22-dimethyldigitoxigenin;
–·–·– (21R)-21,22-dimethyldigitoxigenin.

(C21, O14) conformation is favoured in case of the 22-methyldigitoxigenin.

For disubstituted steroids, the potential-energy difference between (C21, O14) and (C22, O14) conformations is much smaller. The (21R)-21,22-dimethyldigitoxigenin can be expected to have an increased portion in (C21, O14) conformations compared to the (21S) steroid because of i) a diminished energy difference between the conformations in question, ii) a smaller potential-minimum curve of

Table I. Input data and results of MTD calculations.

No.	Steroid	-log H_{50}	Occupied vertices j	δ_7	MTD to S_1^*	MTD to S_2^*	A_1^{theor}	A_2^{theor}
1	3β, 16β-diOMe-GTG	6.47	1, 2, 8, 9, 16	0	0	1	6.40	6.63
2	3β-NH_2-DTG	6.41	1, 16	0	1	2	5.73	5.88
3	16β-OAc-GTG	6.30	1, 8–11, 16	0	1	2	5.73	5.88
4	3β, 16β-diOAc-GTG	5.99	1–4, 8–11, 16	0	1	2	5.73	5.88
5	3β-OAc-DTG	5.91	1–4, 16	0	1	2	5.73	5.88
6	DTG	5.86	1, 16	0	1	2	5.73	5.88
7	3-desoxy-DTG	5.49	16	0	2	3	5.06	5.12
8	DGG	5.31	1, 6, 16	0	2	3	5.06	5.12
9	3β-OAc–22-Me-DTG	5.28	1–4, 13, 16	0	2	3	5.06	5.12
10	22-Me-DTG	5.11	1, 13, 16	0	2	3	5.06	5.12
11	3β-OAc-GTG	5.05	1–4, 8, 16	0	1	3	5.73	5.12
12	GTG	4.97	1, 8, 16	0	1	3	5.73	5.12
13	21ξ, 22-diMe-DTG	4.60	1, 12, 13, 16	0	2	3	5.06	5.12
14	11β-OH-DTG	4.55	1, 5, 16	0	2	3	5.06	5.12
15	17β furanyl compound	4.44	1	0	2	3	5.06	5.12
16	22-allyl-DTG	4.39	1, 13–16	0	3	4	4.39	4.37
17	3β, 16β-diOAc–22-allyl-GTG	4.35	1–4, 8–11, 13–16	0	3	4	4.39	4.37
18	3β-OAc–15β-OH-DTG	4.30	1–4, 7, 16	1	2	3	4.20	4.20
19	15β-OH-DTG	4.10	1, 7, 16	1	2	3	4.20	4.20
20	22-allyl-DGG	<4.00	1, 6, 13–16	0	4	5	3.73	3.62

DTG: digitoxigenin; DGG: digoxigenin; GTG: gitoxigenin; OMe: methoxy; OH: hydroxy; NH_2: amino; OAc: acetoxy; Me: methyl

the (C22, O14) conformation, and iii) the existence of two (C21, O14) conformers. All these conclusions about the relative conformational stability are in excellent agreement with recent interpretations of NMR results [27, 28].

Discussion

In accord with the widely accepted concept of a hydrogen bond between the lactone-ring carbonyl oxygen and the receptor, both determined receptor maps reveal vertex $j = 16$ to be within the receptor cavity. Other common features of S_1^* and S_2^* are the sterically repulsive interactions from the C11β, C12β, and C15β regions of cardiotonic steroids. This is very interesting since digoxin as one of the most often prescribed cardiotonic drugs contains a 12β hydroxyl group which, on the other hand, also decreases the hydrophobic properties.

Besides wall vertex $j = 4$, the 3β substituent displays cavity vertices in the immediate neighbourhood of the C3 atom. In a former MTD investigation [10] using toxicity data for cats, ring A of the steroid backbone was, however, found to be outside of the receptor-interaction area. Apart from ATPase inhibition, this contradiction may be due to the influence of other effects on cardiotoxicity. Actually, the order of the ATPase inhibitory strengths (bufalin>DTG>DGG>GTG>strophanthidin) [18–23] differs from that of cardiotoxicity in cats (bufalin>strophanthidin>DGG>DTG>GTG) [29].

The differences between the receptor maps S_1^* and S_2^* are located in the 16β substituent interpretation. If both the vertex $j = 8$ and $j = 9$ are considered to be within the receptor cavity, just in accord with the single-connected network rule, the repulsive effect of vertex $j = 8$ in S_2^* should be due to intramolecular interactions. It can be derived from X-ray crystal structure data that 16β substituents influence the lactone-ring orientation to some extent. The torsional angle C13–C17–C20–C22 in gitoxin [30], for instance, differs from that in digoxin [31] by 29.6°. The conformationally altered lactone rings should have modified abilities to form receptor hydrogen bonds according to molecular electrostatic potential calculations [7]. Thus, vertex $j = 8$ is imaginable to insert an activity-reducing influence *via* intramolecular interactions although it is located within the receptor cavity sphere.

With regard to the 17β substituent, it is interesting that not only cavity vertices are found in the lactone-ring region. In that connection, the fact is to be underlined that no reference to conformational effects is made in the MTD scheme. The results of the receptor maps should, therefore, be considered together with the potential-energy situation for the conformers in question. As far as the MTD superposition process is concerned, all of the lactone-ring substituted compounds included to construct the hypermolecule (steroids 9, 10, 13, 16, 17, 20) can be assumed to exclusively or partly prefer the (C21, O14) conformation. Thus, the vertex network is suitable in the given manner. However, if the results should be applied to, *e.g.*, C21 monosubstituted compounds, the fact of conformational conversion must be taken into account.

In case that the (C21, O14) conformation is retained in the steroid-receptor complex, the present MTD results suggest a close fit between the receptor enzyme and the unsubstituted steroid in the C22 region whereas the vertex $j = 12$ has no contact with the receptor in correspondence with the MTD findings. Accordingly, vertices $j = 13$, 14 are real wall vertices. Otherwise, if the receptor hydrogen bond to the lactone-ring carbonyl oxygen can only be formed by the adoption of the (C22, O14) conformation, the conformational change would bring vertices $j = 13$, 14, 15 in close proximity to repulsive non-bonded forces of the O14 and C16 regions. The activity-reducing effect is then explained by the conformational activation energy instead of receptor interactions. In this case, the sterically irrelevant vertex $j = 12$ would suggest a larger receptor pocket in this area. However, in view of the potential-energy curves given in Fig. 3 and the fact that for the dimethyl compound 13 a wall vertex ($j = 13$) is occupied, the (C21, O14) conformation is somewhat more likely as it is also concluded from other structure-activity studies on cardiotonic steroids [28, 32, 33].

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