MECHANISM OF ACTION OF A BIOMIMETIC PHENYTOIN RECEPTOR

Saul Wolfe*, Kiyull Yang, Raymond John Bowers, Hee-Sook Shin,and Chang-Kook Sohn

Department of Chemistry, Queen's University, Kingston, Ontario, Canada K7L 3N6

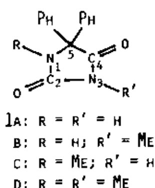
Dedicated to Professor Sir Derek Barton on the occasion of his 70th birthday

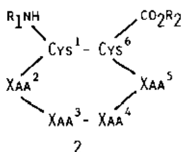
Abstract- The anticonvulsant drug phenytoin (5,5-diphenylhydantoin) is a specific inhibitor of sodium ion transport by the redox-switched cyclodepsipeptide ionophore Boc-L-cystinyl-glycyl-L-prolyl-glycyl-L-prolyl-L-cystine-$OCHPh_2$ (phenceptin), a biomimetic model of a sodium channel receptor. Using various N-methylated analogs of phenytoin and phenceptin, the inhibition is found to involve N1 of the drug and the gly[4]-nitrogen of phenceptin, as predicted by a calculated structure of the 1:1 phenytoin-phenceptin complex.

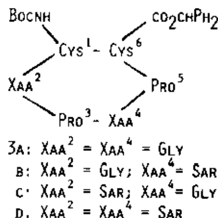
Phenytoin (5,5-diphenylhydantoin) (DPH, 1a), the most widely used drug for the treatment of epilepsy, functions by the specific inactivation of voltage-sensitive sodium channels.[1,2] Although the constitutions of sodium channels are becoming known,[3,4] and several models for their opening and closing have been proposed,[3,5,6] a chemical structure that accounts for the abnormal behavior which triggers a seizure, and the manner in which this structure interacts with phenytoin, have been lacking.

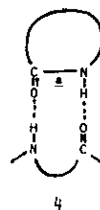
We recently suggested[7] that the opening of a sodium channel which initiates a seizure produces a structure having the ion transport properties of a redox-switched ionophore.[8,9] The simplest biomimetic model that is compatible with this hypothesis, and also with the known constitutions of sodium channels and the requirement for selective binding of sodium ions, is a cyclohexadepsipeptide containing a cystine moiety, e.g., 2.

The search for a sequence Xaa^2-Xaa^5 appropriate for the experimental evaluation of this model was performed using a series of computer programmes developed in our laboratory,[10-12] which have been found to lead to reliable predictions of the solution conformations of peptides, and the energies of intermolecular complexes. This strategy identified 3a, which we have named "phenceptin",[7] as a target for experimental study.









At 37.0 °C, with n-octanol as the membrane phase, phenceptin transports Na^+ (as NaSCN) selectively,[13] at a rate 0.8 that observed with 15-crown-5 under the same conditions.[14] Phenytoin has no effect on sodium transport by 15-crown-5. Likewise, the biologically inactive phenytoin

analog 5,5-*dibenzyl*hydantoin, has no effect on sodium transport by *phenceptin*. In contrast, phenytoin significantly inhibits sodium ion transport by phenceptin,[7] and this is accomplished[7] via the formation of a 1:1 complex between phenytoin and phenceptin, which is a less effective ionophore than phenceptin itself.

These findings provide an opportunity to gain insight into the chemical structure associated with a "drug-receptor interaction", if the nature of the 1:1 phenytoin:phenceptin complex can be determined. This is the objective of the present work.

Figure 1 shows the calculated structure of phenceptin (a stereoscopic viewer is recommended). In this structure, one of the benzene rings lies above the methylene group of gly^2. At 273 K, in $CDCl_3$, these protons are found in the ^1H nmr spectrum at 3.19 ppm. The methylene protons of gly^4 are seen at 3.92 and 4.09 ppm. In n-$C_8D_{17}OH$ solvent, the gly^4 NH shows evidence of hydrogen bonding to a carbonyl oxygen of phenytoin (see Figure 8 of ref. 7), consistent with a part structure 4, in which a is one of the NH-CO bonds of the drug.

As a guide to the projected experimental study, possible structures of 4 were first examined computationally, using the peptide parameters of ref. 10, the hydantoin parameters of ref. 15, and the docking strategy of ref. 12. The energies of all possible complexes between each of the three NH-CO bonds of phenytoin, and NH of gly^4 and each neighbouring CO, or NH of gly^2 and each neighbouring CO, were minimized. The most stable 1:1 complex thus identified is shown in Figure 2. This involves hydrogen bonding between N1-C2 of phenytoin and the NH and CO of gly^4 of phenceptin; in this complex, there are several edge-to-face aromatic-aromatic interactions[16] between the phenyl rings of the two molecules.

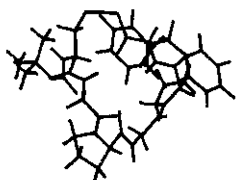
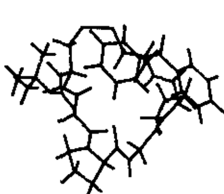



Figure 1

Figure 2

If this structure adequately describes the drug-receptor interaction, the following predictions can be made: (i) 3-methyl-DPH (1b) should have the same effect as phenytoin in the inhibition of sodium ion transport by phenceptin, 1-methyl-DPH (1c) should exhibit reduced inhibition, and 1,3-dimethyl-DPH (1d) should not inhibit; (ii) provided that the conformations of the cyclodepsipeptides remain the same, N-methylation of gly^4 ($gly^4 \rightarrow sar^4$) (3a $\rightarrow$ 3b) should lead to a phenytoin-insensitive ionophore, but N-methylation of gly^2 ($gly^2 \rightarrow sar^2$) (3a $\rightarrow$ 3c) should yield an ionophore resembling phenceptin.

To evaluate these predictions fully, we required the four hydantoins 1a - 1d, and also the four cyclodepsipeptides 3a - 3d. 3-Methyl-DPH (1b) and 1,3-dimethyl-DPH (1d) were prepared from 1a using Me_2SO_4-NaOH.[17] 1-Methyl-DPH (1c) was prepared from 1a by morpholinomethyl protection of N3, alkylation at N1 (MeI, NaH, DMF), and alkaline hydrolysis of the protecting group.[18] Each of the cyclodepsipeptides was obtained by total synthesis, as already described for phenceptin itself,[7] but using sarcosine in place of glycine, as appropriate. All substrates were obtained in analytically pure form.

Kinetic studies were carried out at 37.0 ^{o}C, in a divided beaker transport cell,[7] using n-

octanol as the membrane phase. The external aqueous phase contained NaSCN (20 mM), the internal phase was water, and the membrane phase was 150 μM in ionophore. Transport rates were measured by determination of SCN^- appearance in the internal phase as a function of time; after 12 h, a hydantoin (150 μM) was added and the run was continued for at least 12 h. Figure 3 shows the result obtained using phenceptin (3a) as the ionophore, and 3-methyl-DPH (1b) as the inhibitor. The transport rate decreases from 0.300 μM/h to 0.120 μM/h (60% inhibition).

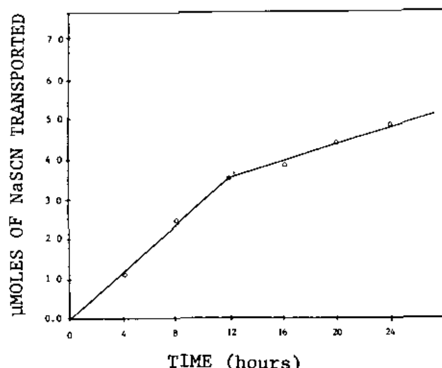


Figure 3

Table 1 summarizes the transport rates and inhibition data; the latter are given relative to phenceptin + phenytoin = 100. The monomethylated analogs 3b and 3c are seen to be comparable to phenceptin as ionophores, and the dimethylated analog 3d is less effective.

Table 1. Effects of Methylated 5,5-Diphenylhydantoins on Sodium Ion Transport by Phenceptin and Methylated Analogs[a]

Ionophore	Transport Rate[b]	Hydantoin[c]	Relative % Inhibition	Ionophore	Transport Rate[b]	Hydantoin	Relative % Inhibition
3a	0.33	none		3b	0.26	none	
		1a	100			1a	11
		1b	97			1b	24
		1c	47			1c	16
		1d	-55			1d	0
3c	0.31	none		3d	0.18	none	
		1a	39			1a	9
		1b	54				
		1c	48				
		1d	16				

[a] See text for concentrations and conditions. [b] In μmol/h; the value listed is the average of at least four determinations. [c] One molar equivalent was added.

In the case of the phenceptin (3a), the inhibition data are consistent with the predictions based on Figure 2: 3-methyl-DPH (1b) is equivalent to phenytoin, 1-methyl-DPH (1c) exhibits less inhibition, and 1,3-dimethyl-DPH (1d) actually *enhances* sodium ion transport (*vide infra*).

With the 4-sarcosyl analog (3b), the results are also consistent with the predictions based on Figure 2: when the nitrogen of gly^4 is blocked, 3b is much less effective than 3a as a phenytoin receptor.

On the other hand, with the nitrogen of gly^2 blocked, sodium ion transport by the phenceptin analog 3c again shows significant response to the addition of phenytoin and its analogs, with the exception of 1,3-dimethyl-DPH. As expected, transport by 3d, the disarcosyl analog of phenceptin,

is virtually unaffected by phenytoin.

The experimental results suggest that 3b has the same backbone conformation as phenceptin, and calculations support this view. The kinetic data also suggest that 3c has a somewhat different conformation; in this case, the structure having the phenceptin backbone conformation is 0.5 kcal/mol higher in energy than the lowest energy conformation found.

We have already noted, in Figure 2, the importance of aromatic-aromatic interactions in the stabilization of the complex involving N1-C2 of phenytoin. Moreover, close inspection of Figure 1 reveals that the movement of ions through the cavity of phenceptin will be hindered by the benzhydryl moiety. These two observations allow the enhancement of sodium ion transport following addition of 1,3-dimethyl-DPH to phenceptin to be interpreted. With this hydantoin, only the aromatic-aromatic interactions with the benzhydryl group are significant; if these interactions cause this group to rotate away from the edge of the cavity in the formation of a complex, the passage of ions through this cavity would be facilitated. Should this interpretation be validated by research in progress, the interaction of aromatic compounds with phenceptin could represent a biomimetic model for the convulsant toxic effects of such compounds.

ACKNOWLEDGEMENTS

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