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Defining determinant molecular properties for the blockade of the apamin-sensitive SK_{Ca} channel in guinea-pig hepatocytes: the influence of polarizability and molecular geometry

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Abstract—QSAR studies of a series of blockers of the SK_{Ca} channel in guinea-pig hepatocytes suggests that the polarizability of the blocker is an important factor controlling the binding to the channel. It is suggested that, upon binding, an ion-pair is formed, a process that is promoted by the reorganization of the water molecules. The polarizability is not adequate to describe the potency of the most potent blockers with a good stereochemical fit to the channel, presumably due to more specific interactions taking place.

Small conductance Ca^{2+}-activated K^+ (SK_{Ca}) channels comprise an important subcategory of K^+ channels.[1,2] They are found in a variety of cell types, both excitable and inexcitable, where they play important physiological roles. Functional, pharmacological and structural data have suggested the existence of subtypes of the SK_{Ca} channel.[3–6] In accordance with these observations, three SK_{Ca} subunits have been identified by DNA cloning, namely SK1, SK2 and SK3.[7,8] We have synthesized several series of blockers of the apamin-sensitive SK_{Ca} channel found in the rat superior cervical ganglion, which it has been suggested is of SK3 type.[9] We have established structure–activity relationships for the blockade of this SK_{Ca} channel subtype.[10–15] However, only a small number of blockers of the apamin-sensitive SK_{Ca} channel in guinea-pig hepatocytes has been reported,[6,16,17] and there is pharmacological evidence to suggest that this K^+ channel is of the SK2 subtype.[18] Thus, it is important to explore the stereoelectronic requirements for the blockade of this putative SK2 channel, since the identification of differential structure–activity relationships for the blockade of the SK2 and SK3 subtypes can guide the design of more selective blockers. In this communication we report preliminary results of a quantitative structure–activity study on the blockers of the apamin-sensitive guinea-pig hepatocyte SK_{Ca} channel.

Apamin, an octadecapeptide from bee venom, blocks the guinea-pig hepatocyte SK_{Ca} channel subtype with an IC_{50} of 1 nM.[16] A number of nonpeptidic blockers have also been described. Their structures are shown in Chart 1, with their potencies presented in Table 1. Among them, the most potent and selective are dequalinium[17] and UCL 1530.[6] So far, no rationalization has been offered for the structure–activity trends in this group of blockers.

We have previously shown that an electronic effect operates in the blockade of the SK_{Ca} channel in rat sympathetic neurons (SK3 subtype)[9] by dequalinium analogues, since the blocking potency correlates with the energy of the LUMO (E_{LUMO}) of the compound.[10,11] However, in the present series of blockers, there is no correlation of the potency with E_{LUMO} ($r = 0.49$), E_{HOMO} ($r = 0.07$) or the energetic gap between HOMO and LUMO ($r = 0.20$) calculated using the AM1 Hamiltonian.[19] Similarly, the dipole moment of the compounds, calculated at the ab-initio STO-3G level of theory, does not correlate with their potency ($r = 0.17$).

Keywords: Polarizability; QSAR; SK_{Ca} blockers; Apamin; UCL 1848.
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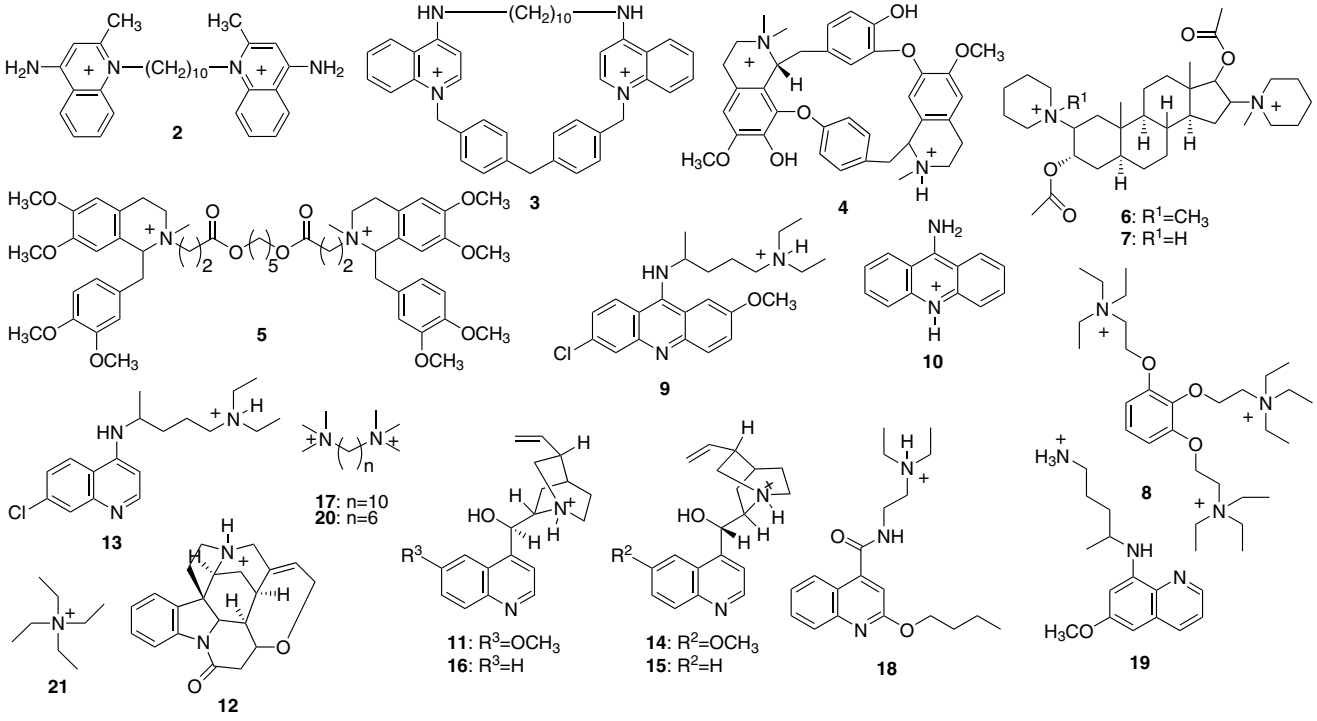

Chart 1. Chemical structures of the SK_{Ca} channel blockers.

Table 1. Pharmacological results and values for the parameters used in the study

Compound	Name	IC_{50} (μM)	α^{d} (Å^3)	V^{e} (Å^3)	spc_α^{f}
1	Apamin	0.001[a]	NC[g]	NC[g]	NC[g]
2	Dequalinium	1.7[b]	31.19	569.85	0.0547
3	UCL 1530	2.6[b]	43.11	749.25	0.0575
4	*d*-Tubocurarine	3[a]	38.14	680.57	0.0560
5	Atracurium	3[a]	53.49	1077.00	0.0497
6	Pancuronium	3.5[a]	30.85	691.30	0.0446
7	Vecuronium	4.5[c]	30.43	674.92	0.0451
8	Gallamine	12[b]	29.41	675.05	0.0436
9	Quinacrine	73[a]	25.82	476.04	0.0542
10	9-Aminoacridine	120[a]	14.46	225.08	0.0642
11	Quinine	150[a]	19.49	381.10	0.0511
12	Strychnine	190[a]	19.22	364.98	0.0527
13	Chloroquine	200[a]	18.76	390.81	0.0480
14	Quinidine	240[a]	19.56	381.27	0.0513
15	Cinchonine	370[a]	17.71	350.88	0.0505
16	Cinchonidine	420[a]	17.73	350.70	0.0506
17	Decamethonium	450[a]	14.74	382.12	0.0386
18	Dibucaine	470[a]	20.60	427.89	0.0481
19	Primaquine	970[a]	16.06	322.91	0.0497
20	Hexamethonium	2000[a]	11.39	299.97	0.0380
21	TEA	7900[a]	7.23	196.50	0.0368

[a] Data from Ref. 16.
[b] Data from Ref. 6.
[c] Data from Ref. 17.
[d] Mean alpha polarizability of the molecule.
[e] Molecular volume.
[f] Specific polarizability.
[g] Not calculated.

Another fundamental property[20] that relates to the electronic structure of the molecule as a whole is polarizability.[21] To investigate whether there is a dependence of potency on the polarizability of the molecule, the mean alpha polarizabilities (α) of compounds **2**–**21** were calculated[33] and are presented in Table 1. Due to computational limitations arising from the size of apamin, the α value for this peptide was not

calculated. Linear regression analysis of $-\log(IC_{50})$ (pIC_{50}) versus α yielded Eq. 1:

$$\begin{aligned} pIC_{50} &= 0.086(\pm 0.011)\alpha + 2.06(\pm 0.28) \\ n &= 20, \quad r = 0.89, \quad r^2 = 0.79, \\ s &= 0.522, \quad F = 66.48, \end{aligned} \tag{1}$$

where n is the number of compounds, r is the correlation coefficient, s is the standard error of the estimate and F is the Fischer variance ratio. Eq. 1 suggests that molecules which are more easily polarized interact with the channel protein more efficiently.

This set of blockers comprises a rather heterogeneous group of structures, having either one or two positively charged N atoms, the latter being either quaternary or tertiary, embedded in aliphatic or aromatic systems (Chart 1). Furthermore, the flexibility of the molecules varies substantially. Despite this structural variability, the potency of the compounds as blockers of the apamin-sensitive SK_{Ca} channel seems to be a function of a single molecular property, polarizability. The QSAR model based on Eq. 1 implies that the blockade of the channel does not depend upon the interactions of specific functional groups of the blockers with the channel.

The binding of these blockers probably occurs at a polar anionic site, since it has been shown that Asp330 and Asn357 located in the pore of the SK2 channel, play an important role in the binding of *d*-tubocurarine (**4**).[22] Since the functional SK_{Ca} channel is believed to be composed of four SK2 subunits, each contributing an Asp330 residue, it is likely that the molecule of the blocker senses a strong negative electrostatic field as it approaches the binding site. The polarizability reflects the ease of distortion of the electron cloud of the molecule, due to the application of an external electrostatic field. However, the polarizability contains both an electronic and a steric component.[21] An attempt was made to separate the steric from the electronic part of polarizability by computing the molecular volume (V) of the compounds (using SPARTAN)[23] and by dividing the polarizability α by the volume of the molecule to give the specific polarizability (spc_a). The correlations of pIC_{50} with volume (Eq. 2) or spc_a ($r = 0.41$, equation not shown) alone are worse than QSAR Eq. 1. Hansch et al. have pointed out that although the polarizability is usually regarded as a volume related parameter, in many cases its replacement by volume in QSAR studies results in poorer correlations.[21]

$$\begin{aligned} pIC_{50} &= 0.00435(\pm 0.001)V + 2.01(\pm 0.33) \\ n &= 20, \quad r = 0.85, \quad r^2 = 0.73, \\ s &= 0.589, \quad F = 48.34. \end{aligned} \tag{2}$$

A plot of pIC_{50} versus the mean polarizability α is presented in Figure 1. There are two major outliers on the plot. The blocking potency of dequalinium (**2**) is under predicted by approximately 1 order of magnitude while that of atracurium is over predicted by approximately an equal amount. It is noteworthy that atracurium (**5**) bears a long linker, with 14 rotatable bonds, connecting the two quaternary N atoms, a fact that might introduce unfavourable entropic factors in the binding of the molecule to the channel. The higher activity of dequalinium than predicted by Eq. 1 may be attributed to the presence of the 4-aminoquinolinium groups in the molecule. We have previously shown that the 4-aminoquinolinium moiety is superior than many other heterocyclic and alkylammonium groups for blockade of the SK3 channel in rat superior cervical ganglion neurons.[24]

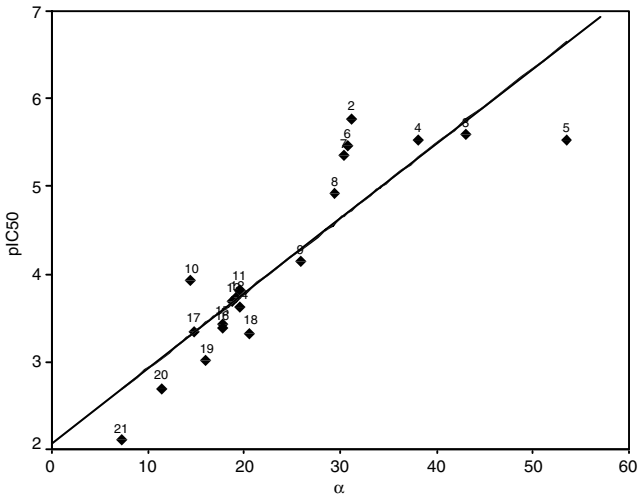

Figure 1. Plot of pIC_{50} versus α for the compounds of Table 1.

It is appropriate to attempt to assign a physical explanation for the dependence of the binding strength of the compounds on their polarizability. The most potent of the molecules (Chart 1) bear the quaternary N atoms embedded in a hydrocarbon environment, where the positive charge is highly dispersed on the vicinal H atoms (data not shown). Since the binding site is conceived as being in the outer part of the pore of the channel, it is likely that the binding occurs in an aqueous environment. Thus, the dehydration (desolvation) of the charged molecules may be an important factor for binding. However, no correlation was established between pIC_{50} and the calculated hydration enthalpies of the compounds using the AM1–SM2.1[25,26] methodology (data not shown). It was mentioned above that these positively charged compounds probably interact with an anionic site formed by Asp330 of the SK2 protein. This association can be regarded as the formation of a large ion-pair. Diamond has introduced the concept of water structure-enforced ion-pairing for large ions, including the tetraalkylammonium ions.[27] Such an association has the opposite type of dependence on charge and size compared with electrostatic ion-pairing. Although electrostatic forces do contribute, this type of association is primarily not due to an electrostatic ion–ion interaction but, rather, it is forced by the water itself in trying to minimize the disturbance to its structure and to maximize the entropy of the system. The magnitude of this effect increases as the ions get larger and should not occur with highly charged ions which interact

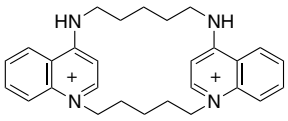

Chart 2. Chemical structure of UCL 1848.

strongly with water.[27] The SK_{Ca} blockers of the present study fulfill these two criteria as they are large molecules having their positive charges significantly dispersed.

Although this study has revealed the importance of polarizability as a determinant of the potency of some agents that block SK2 channels, other factors are likely to contribute. There is already some suggestion in the results of Figure 1 that there may be a 'ceiling', at about 30 $Å^3$, for the influence of polarizability. In keeping with this, the greater than predicted potency of dequalinium (**2**) suggests that structural and electronic factors are coming into play, as already discussed. Indeed, by taking dequalinium as a lead compound, it has been possible to discover blocking agents that are much more active and selective. An example is UCL 1848 (Chart 2), which has been shown to inhibit ^{125}I-apamin binding to intact guinea-pig hepatocytes with a K_i of 0.14 ± 0.01 nM,[18] corresponding to a calculated[16] IC_{50} value of ca. 0.19 nM. The calculated mean α polarizability of UCL 1848 is 28.99 $Å^3$, which on the basis of Eq. 1, gives a predicted IC_{50} value of ca. 28 μM, 5 orders of magnitude higher than the experimentally determined potency. Thus, the polarizability alone cannot explain the potency of blockers that have a good stereochemical fit to the binding site. This consideration also applies to a recent study[28] relating SK blocking activity to molecular mass, volume and diffusivity.

In conclusion, QSAR analysis of a set of compounds known to block the apamin-sensitive SK_{Ca} channel in guinea-pig hepatocytes has revealed that the process of binding of the compounds to the channel is influenced by the polarizability of the molecules. It is suggested that the mechanism of block involves the formation of a large ion-pair between the compound and the channel protein. Polarizability should be further explored as a potentially useful parameter for the quantification of the interactions of large organic ions (including charged peptides) in an aqueous environment. However, the activity of highly potent blockers such as UCL 1848, forming specific interactions with the channel, cannot be accounted for by the polarizability alone. The precise shape and electronic characteristics of the molecule then become the dominant factors.

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33. A conformational search was performed on molecules **2**–**21** using the XED/COSMIC molecular modeling software.[29,30] The global minimum energy conformer of each compound was subjected to full geometry optimization at the ab-initio level using the PC GAMESS version[31] of the GAMESS (US) quantum mechanics package[32] and the STO-3G basis set. The mean alpha polarizability was then calculated for the optimized structure using the same basis set.