Synthesis and Pharmacological Testing of Dequalinium Analogues as Blockers of the Apamin-Sensitive Ca^{2+}-Activated K^+ Channel: Variation of the Length of the Alkylene Chain

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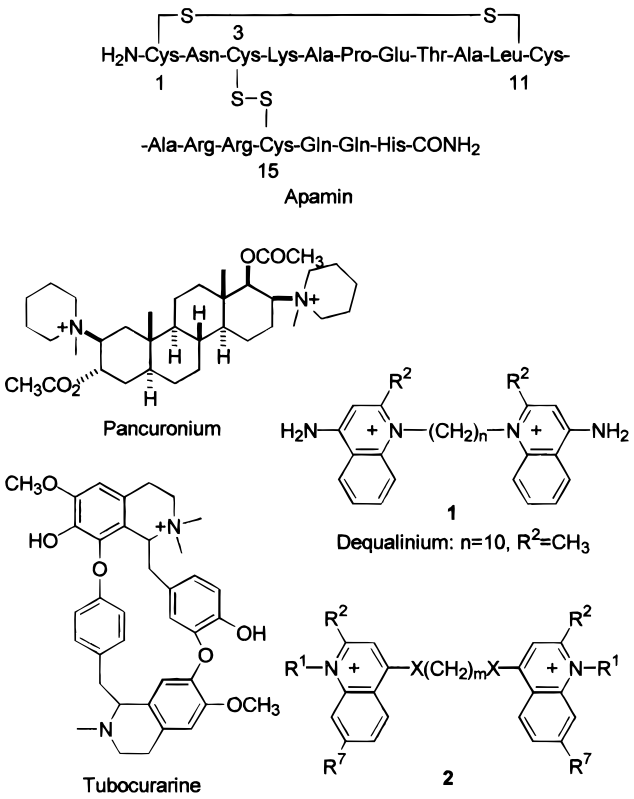
Dequalinium is a potent and selective blocker of the small conductance Ca^{2+}-activated K^+ (SK_{Ca}) channel in rat sympathetic neurones. Analogues of dequalinium possessing 3−6, 8, 10, and 12 methylene groups in the linking chain have been synthesized and tested for inhibition of the afterhyperpolarization in rat sympathetic neurones. The compounds having a 5−12-carbon chain showed very little variation in their activity as SK_{Ca} channel blockers. The analogues possessing four and three methylenes exhibited 3- and 8-fold lower potency, respectively, compared with dequalinium. These results are discussed in the context of possible modes of binding of the compounds to the SK_{Ca} channel.

Introduction

The small conductance Ca^{2+}-activated K^+ (SK_{Ca}) channel is an important but relatively little studied subtype,[1,2] and this is mainly due to the lack of readily accessible, potent blockers. The currently available blockers can be broadly divided into two classes: (i) Natural peptidic toxins, such as apamin[3−5] (Chart 1), leiurotoxin I[6] (scyllatoxin), and PO5,[7] acting at the low nanomolar range and (ii) synthetic compounds bearing two positively charged groups, such as atracurium, tubocurarine, and pancuronium[8−10] (Chart 1), having activities in the micromolar range. The relatively low potency of these compounds and difficulty of obtaining the pure toxins present a need for the development of potent, synthetic SK_{Ca} channel blockers. Such agents will be valuable pharmacological tools for the study of the physiology and pathophysiology of SK_{Ca} channels and may also have therapeutic applications, since there is evidence for the involvement of this channel in conditions such as myotonic muscular dystrophy[11−13] and EtOH intoxication.[14]

Dequalinium (**1**, $n = 10$, $R^2 = CH_3$, Chart 1) has recently been shown to be a potent and selective blocker of the SK_{Ca} channel in rat sympathetic neurones,[15,16] and we have initiated studies toward identifying the pharmacophore of dequalinium for SK_{Ca} channel blockade,[17−21] with the aim of designing more potent compounds. The 2-Me group of this compound makes little contribution to SK_{Ca} channel blockade,[19] whereas the 4-NH_2 group contributes substantially and its role has been suggested to be electronic, probably via delocalization of the positive charge of the ring.[19] Furthermore, good correlations have been obtained between the blocking potency of dequalinium analogues and the energy of the LUMO.[20,21] The quinolinium groups of dequalinium have been replaced by other charged heterocycles in an effort to elucidate their role. The quinolinium group was found to give the optimum blocking potency, and we suggested that this may arise from the ring-shaped electrostatic field around this group.[18]

Chart 1



Another structural feature of dequalinium that merits investigation is the 10-methylene chain joining the two quinolinium groups. Very little is known about the contribution of this linker to SK_{Ca} blockade, although it has been shown that restriction of the conformational mobility of the chain via introduction of two triple bonds resulted in a 2-fold loss in potency.[18] Furthermore, in dequalinium analogues of the general structure **2** (Chart 1), having inverted quinolinium groups, extension of the aliphatic chain to 12 carbons or reduction to 8 carbons

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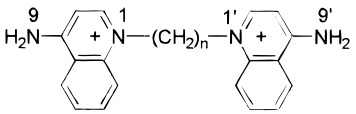
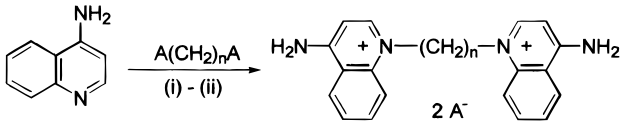
Table 1. Calculated Internitrogen Distances for the Compounds and SK_{Ca} Channel Blocking Activities



compd	n	$N^1-N^{1'}$ (Å)	$N^9-N^{9'}$ (Å)	$IC_{50} \pm SD$ (μM)	EMR[a] ± SD	r[b]
dequalinium		13.8	21.4	0.9 ± 0.2	1	15
3	3	4.9	11.8	4.5 ± 0.7	8.3 ± 3.6	3
4	4	6.2	14.1	1.8 ± 0.3	3.2 ± 1.3	3
5	5	7.4	14.4	1.0 ± 0.2	1.0 ± 0.2	5
6	6	8.7	16.4	2.0 ± 0.1	1.8 ± 0.6	3
7	8	11.2	18.7	2.0 ± 0.3	2.1 ± 0.8	3
8	10	13.7	21.2	1.4 ± 0.3[c]	1.3 ± 0.5[c]	7
9	12	16.2	23.6	1.7 ± 0.5	1.8 ± 0.5	5

[a] Equieffective molar ratio: the ratio of the concentrations of the test compound and dequalinium that cause 50% inhibition of the AHP, as determined in the same experiment. [b] Number of neurons tested. [c] Data from ref 19.

Scheme 1[a]



3 - 7, 9

[a] Methods: (i) **3**, **4**, A = I, EtOH, reflux, 96 h; (ii) **5**−**7**, A = I, MEK, reflux, 96 h; (iii) **9**, A = Br, MEK, reflux, 96 h.

produced a small decrease in potency.[21] In the present work, compounds of the general structure **1** (R^2 = H) have been synthesized in which the length of the alkylene chain has been systematically varied from 3 to 12 methylene groups. The effect of this structural modification on the ability of the compounds to block the slow afterhyperpolarization (AHP, mediated by the opening of SK_{Ca} channels) in rat sympathetic neurones has been examined. The 2-Me group present in the molecule of dequalinium has been omitted in the current series of analogues because its presence results in synthetic difficulties[19,20] but does not contribute significantly to blocking activity.

Chemistry

The compounds were prepared via reaction of 4-aminoquinoline[22] with the respective dihalide (Scheme 1). Methyl ethyl ketone (MEK) was used as the solvent in the synthesis of the analogues with n = 5−12. For the three- and four-methylene homologues, however, a more polar solvent (EtOH) had to be employed to facilitate the dissolution of the intermediate 1-(ω-iodoalkyl)-quinolinium salt formed in the course of the reaction. When n = 3 or 4, these monoquinolinium salts had insufficient solubility in MEK to allow them to react further to yield the final compounds. Furthermore, we were unable to obtain the two-methylene analogue, as reaction of 4-aminoquinoline with either 1,2-dibromoethane or 1,2-diiodoethane gave only the 4-amino-1-vinylquinolinium salts. Compound **8** has been reported previously.[19]

Biological Testing

The potency of compounds as blockers of the SK_{Ca} channel was determined from their ability to inhibit the afterhyperpolarization which follows the action potential in rat sympathetic neurones as described previously.[16] Briefly, intracellular recordings were made from rat superior cervical ganglion neurons maintained in tissue culture for 3−10 days. Action potentials were evoked every 5 s by injection of depolarizing current pulses, and drugs were applied at known concentration in the continually flowing bathing solution. The amplitude of the AHP was measured in the absence and presence of the test compounds to determine the percentage inhibition. Dequalinium was tested on all cells, and equieffective molar ratios (EMR), i.e. the ratio of the concentrations of the test compound and dequalinium that cause 50% inhibition of the AHP, as determined in the same experiment, have been presented to allow for any variation in sensitivity during the course of the study. IC_{50} and EMR values were determined by simultaneous nonlinear least-squares fitting of the Hill equation to the data. The fitting procedure used provides estimates for the IC_{50} and EMR values for the blocker ± an approximate standard deviation. Each concentration of drug was tested on at least three neurons.

Results and Discussion

Biological results for the compounds are given in Table 1. Clearly, variation of the number of methylene groups in the alkylene chain from 5 to 12 has little effect on the SK_{Ca} channel blocking activity of the compounds. However, the four- and three-carbon homologues show a 3- and 8-fold reduction in potency, respectively. These results should be examined in the context of the structural requirements for SK_{Ca} channel blockade.

Apamin (Chart 1) contains two contiguous arginine residues at positions 13 and 14, the charged guanidinium groups of which are believed to be part of the pharmacophore, although, alone, they cannot account for the potency of apamin.[23] Several studies on the tertiary structure of apamin exist in the literature, the most recent of which involved 2D NMR and distance geometry calculations.[24] The lack of nuclear Overhauser effects arising from the side chains of Arg_{13} and Arg_{14} suggests that these are mobile in solution and, therefore, the distance between the two charged guanidinium groups cannot be estimated with certainty.

On the other hand, the discovery that tubocurarine and pancuronium (Chart 1), which possess two charged N atoms rigidly held at a distance of ~11 Å, are effective blockers of the SK_{Ca} channel led to the suggestion that the pharmacophore for SK_{Ca} channel blockade incorporates two charged groups at such a distance.[9]

Table 1 shows the distance between the two quaternary N atoms in the present series of compounds. The

distances were measured with the alkylene chain adopting the extended (fully trans) conformation and thus refer to the maximum possible separation. They were calculated using the XED/COSMIC molecular modeling system.[25,26] It is evident that the blocking potency of the compounds is remarkably insensitive to the maximum distance $N^1-N^{1'}$ between the quinolinium N atoms. Thus, analogues in which the distance varies from 7 to 16 Å have essentially the same potency. Further reduction in the distance to ~6 and ~5 Å causes some loss in potency. These results are very different to the well-known critical dependence on chain length of the ganglion blocking and neuromuscular blocking effects of alkyl bis-trimethylammonium compounds,[27] and they raise a number of questions about the way in which these dequalinium analogues interact with the K^+ channel. Previous results[18] (and unpublished observations) indicate that both quinolinium groups are important for SK_{Ca} channel blocking activity, and consequently the spacing of the two groups can be expected to be important for activity. However, there is a potential complication in that molecular orbital calculations show that the positive charge of the quinolinium group is extensively delocalized over the H atoms,[18] and the increase in potency associated with the presence of an amino substituent at position 4 of the quinolinium ring has been attributed to even greater delocalisation of the positive charge.[19,20] Consequently, there may be considerable tolerance in the spacing of the quinolinium groups, and it may be misleading in this situation to use interatomic distances as a variable in SAR studies.

Furthermore, it is likely that the binding site recognizes the electrostatic field around the quinolinium group and not individual partial atomic charges. Thus, it has been suggested that the superiority of the quinolinium ring for SK_{Ca} channel block over alkylammonium groups may be due to the difference in their electrostatic potential maps.[18] The field around the quinolinium group being larger and ring-shaped may interact more favorably with rings of negative charge known to be present in many cation channels. Under these circumstances, the conformational mobility of the alkylene chain of the molecule together with the presence of large positive fields at either end of the chain might render the molecule capable of favorable ionic contacts over a range of chain lengths, provided they exceed some minimum value.

The above discussion assumes that drug binding is to an anionic site. However, it has been suggested that dequalinium and analogues may bind at a site containing aromatic groups.[18] In this latter case, the insensitivity of the strength of binding in relation to the alkylene chain length may be explained in terms of a folded or partially folded "active" conformation, provided that there is no severe steric interference between the binding site and various gauche forms of the alkane chain. It is clear from the results obtained with this series that activity is optimal when the chain length has reached five methylene groups. Compounds having more methylene groups would still be able to fold and adopt the correct conformation, while reduction in the length of the chain may introduce energy penalties in the folding process, resulting for example from the presence of eclipsed H atoms in the chain. Such unfavorable conformational energy factors would be expected to reduce the free energy of the binding process. This hypothesis would also be applicable to binding at an anionic site.

Conclusion

The dependence of the SK_{Ca} channel blocking potency of dequalinium analogues on the length of the alkylene chain has been systematically investigated. It has been found that compounds with 5−12 methylene groups have similar potencies. Reduction of the chain length below five carbon atoms results in some loss of potency. The results may be accounted for in terms of the flexibility of the linker, extensive delocalization of the positive charge of the quinolinium ring, and the possible existence of a folded active conformation of the molecule.

Experimental Section

Chemistry. Nuclear magnetic resonance (NMR) spectra were recorded on a Varian VXR-400 (400 MHz) spectrometer in DMSO-d_6, and mass spectra were run on a ZAB SE or VG 7070H spectrometer. The structures of all compounds were consistent with their respective NMR and mass spectra. The purity of the compounds was assessed by analytical reverse phase high-performance liquid chromatography (HPLC) on either a Gilson or a Shimadzu HPLC apparatus with a UV detector at 215 nm and a Kromasil C18 7 μm column. Isocratic elutions using a solvent mixture of A (water + 0.1% TFA) and B (MeOH + 0.1% TFA) were performed at a flow rate of 1 mL/min. The ratio of A:B is indicated in the experimental data. In each case, the major peak corresponding to the compound represented at least 97.2% of the absorption at 215 nm.

General Procedure for the Preparation of 3 and 4. 4-Aminoquinoline[22] (0.2 g, 0.14 mmol) and the corresponding diiodoalkane (0.07 mmol) in EtOH (15 mL) were heated under reflux for 96 h under an inert atmosphere (Ar) and in the absence of light. The solid was collected by filtration and washed extensively with Et_2O. The cream-colored solid was dried (40 °C, 0.1 mmHg) for 24 h to afford the product.

1,1′-(Propane-1,3-diyl)bis(4-aminoquinolinium) diiodide (3): yield 29%; mp >300 °C; HPLC A:B = 70:30. Anal. ($C_{21}H_{22}N_4I_2$) C, H, N.

1,1′-(Butane-1,4-diyl)bis(4-aminoquinolinium) diiodide (4): yield 53%; mp >300 °C; HPLC A:B = 70:30. Anal. ($C_{22}H_{24}N_4I_2$) C, H, N.

General Procedure for the Preparation of 5−7 and 9. 4-Aminoquinoline[22] (0.2 g, 0.14 mmol) and the corresponding dihaloalkane (0.07 mmol) in MEK (10 mL) were heated under reflux for 96 h under an inert atmosphere (Ar). The solid was collected, washed thoroughly with MEK, and recrystallized from EtOH/MeOH.

1,1′-(Pentane-1,5-diyl)bis(4-aminoquinolinium) diiodide (5): recrystallization from H_2O; yield 49%; mp >300 °C; HPLC A:B = 65:35. Anal. ($C_{23}H_{26}N_4I_2 \cdot 0.8H_2O$) C, H, N.

1,1′-(Hexane-1,6-diyl)bis(4-aminoquinolinium) diiodide (6): yield 58%; mp 275−276 °C; HPLC A:B = 60:40. Anal. ($C_{24}H_{28}N_4I_2 \cdot 0.8CH_3OH$) C, H, N.

1,1′-(Octane-1,8-diyl)bis(4-aminoquinolinium) diiodide (7): yield 57%; mp 278−279 °C; HPLC A:B = 60:40. Anal. ($C_{24}H_{28}N_4I_2 \cdot 0.8CH_3OH$) C, H, N.

1,1′-(Dodecane-1,12-diyl)bis(4-aminoquinolinium) dibromide hemihydrate (9): yield 36%; mp 267−268 °C; HPLC A:B = 45:55. Anal. ($C_{30}H_{40}N_4Br_2 \cdot 0.5H_2O$) C, H, N.

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Supporting Information Available: ^1H NMR and mass spectral data for compounds **3**−**7** and **9** (2 pages). Ordering information is given on any current masthead page.

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