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Original article

Homologation of mexiletine alkyl chain and stereoselective blockade of skeletal muscle sodium channels

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Abstract – The optical isomers (-)-(*S*)- and (+)-(*R*)-3-(2,6-dimethylphenoxy)-2-methyl-1-propanamine (Me2), homologues of the antiarrhythmic and antimyotonic drug mexiletine (Mex), were synthesized and assayed as new potential antimyotonic agents. As observed with Mex, Me2 exhibits an enantioselective behaviour. Tests carried out on sodium currents of single muscle fibres of *Rana esculenta* demonstrated that (-)-(*S*)- and (+)-(*R*)-Me2 were less potent than Mex in producing tonic block, but showed a higher use-dependent block. (-)-(*S*)-Me2 and (-)-(*R*)-Mex were also used to study the excitability of muscle fibres of myotonic ADR mice, a phenotype of a recessive form of low G_{Cl} myotonia. (-)-(*S*)-Me2 reduced spontaneous discharges and after discharges better than (-)-(*R*)-Mex in agreement with the use-dependent block of sodium currents. © 2000 Éditions scientifiques et médicales Elsevier SAS

antiarrhythmic drug / mexiletine / chiral analogues / myotonia / sodium channel tonic block / sodium channel use-dependent block

1. Introduction

Mexiletine [1-(2,6-dimethylphenoxy)-2-propanamine] (Mex) (*figure 1*) is an established antiarrhythmic drug belonging to the IB class [1].

The pharmacodynamic theory, common for local anaesthetic-like drugs, provides for the block of voltage-gated sodium channels [2] through interaction with segment 6 of domain IV of the sodium channel α-subunit [3]. According to the 'modulated receptor' hypothesis [4, 5], the affinity for sodium channel receptors increases in those channel conformations (open and/or inactivated) favoured by high frequency of stimulation [2, 6]. This behaviour leads to the so-called 'use-dependent' or phasic block and is due to an accumulation of blocking on depending the slow recovery from inactivation of the drug-bound channels during membrane repolarization [2, 3, 7, 8]. The use-dependency of mexiletine action supports its selectivity towards the arrhythmic heart; all the same, mexiletine has a narrow therapeutic ratio, many adverse effects (mainly cardiovascular and nervous) being dose-related and sometimes severe enough to necessitate discontinuation of therapy [9].

A few years ago, by virtue of its pharmacokinetic properties [10] and, above all, of its mechanism of action, Mex was clinically proposed in the symptomatic treatment of myotonic syndromes, which comprise a series of dominant and recessive genetic channelopathies of skeletal muscle characterized by membrane hyperexcitability and delayed muscle relaxation after voluntary contrac-

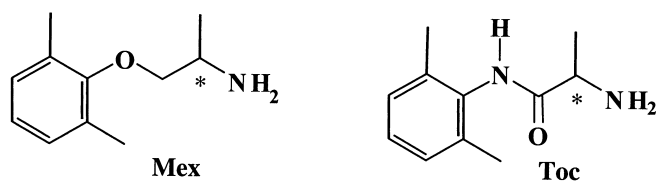

Figure 1. Structures of mexiletine (Mex) and tocainide (Toc).

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tion [11]. Mexiletine has also been employed in some hereditary non-dystrophic myotonias [12]: myotonia congenita (chloride channel disease) [11] and paramyotonia congenita (sodium channel disease) [13]. When administering Mexiletine as an antimyotonic drug, doses are as large as those used in the antiarrhythmic treatment. Thus the antimyotonic therapy, which has to be prolonged through the whole patient's life, might elicit adverse reactions similar to those observed during the antiarrhythmic therapy [11].

Conceivably, mexiletine analogues provided with higher use-dependency of action should present a wider therapeutic ratio, being more selectively active on hyperexcited tissues, i.e. arrhythmic heart and myotonic muscle fibres. As part of a program aimed at preparing use-dependent sodium channel blockers useful as antimyotonic drugs [14], we started from the observations in favour of a common stereospecific domain [15] for Mex and tocainide (Toc), an α-amino xylidide belonging to the IB antiarrhythmic class (see structure in *figure 1*), in the pore of Na^+ channels [3]: some hints might be extrapolated from the α-amino xylidide series structure–activity relationships (SAR) [16] and applied these to the relatively less explored xylyloxy alkyl amines, such as Mex. Thus, β-homologation of Toc improved pharmacokinetic and pharmacodynamic properties [17] and this was rationalized invoking an increased basicity which, in turn, would both favour the presence of the drug in charged form in the biophase and discriminate the passage through the blood–brain barrier, so diminishing the side effects on the central nervous system. On the other hand, an increased lipophilicity, that may be supposed to be caused by the homologation, does favour the access to the cytoplasmic side of the membrane where the binding site is accepted to be located [18–20]: the drugs are normally applied outside the cell and the increased lipophilicity would facilitate the diffusion through the membrane, increasing the amount of drug that has access to the binding site [21]. Thus we speculated that, given the bioisosteric relationship holding between Toc and Mex, and the similarities between heart and muscle sodium channel isoforms which make both Toc and Mex valuable antiarrhythmic as well as antimyotonic drugs, the homologation would improve, once again, the pharmacokinetic and pharmacodynamic profile of the latter.

As expected, the calculated pK_a and LogP values [22] for Me2 (9.7 ± 0.1 and 2.8 ± 0.2, respectively) were higher than the corresponding values for Mex (8.6 ± 0.1, and 2.2 ± 0.2, exp. 2.15 [23], respectively). Therefore, in this work we homologated Mex, moving the amino group away from the stereogenic centre by insertion of a methylenic group. Given that in vitro [15] and in vivo [24] studies on cardiac muscle cells evidenced a stereoselective behaviour of optical isomers of Mex, being the (-)-(*R*)- enantiomer more potent than the (+)-(*S*)- one and that a stereoselective site for sodium channel blockers on adult skeletal muscle fibres has been evidenced too [3, 15], we prepared the optical isomers (-)-(*S*)- and (+)-(*R*)-3-(2,6-dimethylphenoxy)-2-methyl-1-propanamine (Me2) (*figure 2*). The effects of Me2 on sodium currents of single frog muscle fibres, and the ability to suppress the pathological hyperexcitability of skeletal muscle isolated from myotonic arrested development of righting response (ADR) mice, a phenotype of a recessive form of low G_{Cl} myotonia [25–27], were evaluated. The stereoselectivity of action of Me2 enantiomers in comparison to that of Mex enantiomers was evaluated.

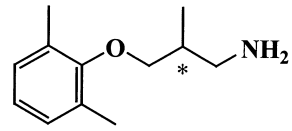

Figure 2. Structure of 3-(2,6-dimethylphenoxy)-2-methyl-1-propanamine (Me2).

2. Chemistry

Mex homologues (**5a–b**) were obtained through the stereospecific synthesis described in *figure 3*, starting from (-)-(*R*)- and (+)-(*S*)-3-bromo-2-methyl-1-propanol (**1a–b**); **1a** and **1b** were converted to the corresponding phthalimido alkanols (**2a–b**) by reaction with potassium phthalimide. The halogenation was led, slightly modifying reaction conditions used in the past to convert benzyl alcohols into the corresponding bromides, in good to low yields [28]: the modified procedure gave **3a–d** in high yields. Compounds **3a–d** were coupled with 2,6-dimethylphenol to give the xylyloxy derivatives **4a–b** [29]. The target compounds **5a–b** were obtained by deprotection of the amino group with N_2H_4 [30] and, finally, conversion into their hydrochloride salts [(-)-(*S*)- and (+)-(*R*)-3-(2,6-dimethylphenoxy)-2-methyl-1-propanamine (Me2)]. The enantiomeric purity (e.p.) of **5a** and **5b** was evaluated by HPLC analysis of the corresponding *N*-acetyl derivatives (**6a** and **6b**) on chiral stationary phase (CSP) and it was 99% and 97%, respectively; [CSP: CHIRALCEL OD, (DAICEL Co.), mobile phase: n-hexane/i-PrOH = 95/5, flow rate: 1.0 mL/min]. (-)-(*R*)- and (+)-(*S*)-Mex were obtained by optical resolution of a racemic mixture and the corresponding e.p. was evaluated as previously described [31].

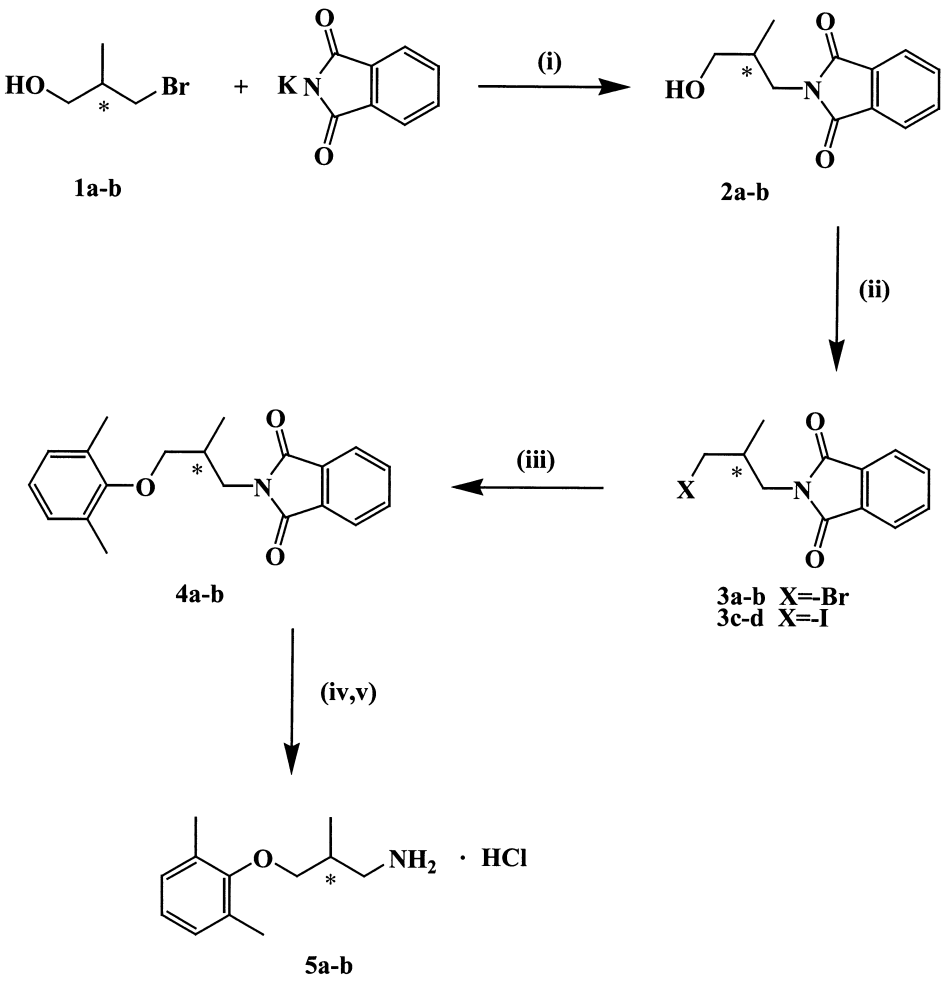

Figure 3. Conditions: (i) DMF, 70–80 °C, 24 h; (ii) HX, toluene –5 °C → room temperature, 5 h; (iii) 2,6-dimethylphenol, NaH, DMF, room temperature, 3 h; (iv) N_2H_4 AcOH, EtOH, reflux, 1.5 h; (v) HCl, Et_2O, room temperature.

3. Pharmacology

3.1. Effects of Mex and its analogue on sodium currents of single muscle fibres

The tonic block exerted by in vitro application of the test compounds was calculated as percent reduction of the inward sodium transient current ($I_{Na\ max}$) elicited by infrequent depolarizing stimulation from the holding potential of –100 to –20 mV to evaluate drug interaction with the channels at the holding potential, i.e. in the resting state. Both Mex and Me2 produced a block of $I_{Na\ max}$ in this condition (*figure 4*), in a concentration-dependent manner. As already observed with Mex [14], the effects of Me2 were stereoselective. *Figure 5* shows the concentration–response curves of (-)-(*S*)- and (+)-(*R*)-enantiomers of Me2 in comparison with that of (-)-(*R*)-Mex, the eutomer of Mex. (-)-(*S*)-Me2 was more potent than its enantiomer, both being less potent than (-)-(*R*)-Mex in producing the tonic block of $I_{Na\ max}$. The calculation of the half-maximal concentrations (*figure 6*) confirmed the stereoselective behaviour; in fact the eudismic ratios [IC_{50}distomer/IC_{50} eutomer] were 2 and 1.7 for Mex and Me2, respectively. In *figure 6* it is also shown that Mex was about twice as potent as Me2 in producing the tonic block. The use-dependent behaviour of each compound was evaluated with trains of test pulses from the h.p. to –20 mV at the frequency of 2 and 10 Hz. With this protocol in the presence, but not in the absence, of the test compounds, a further reduction in peak sodium current over the tonic block was observed that progres-

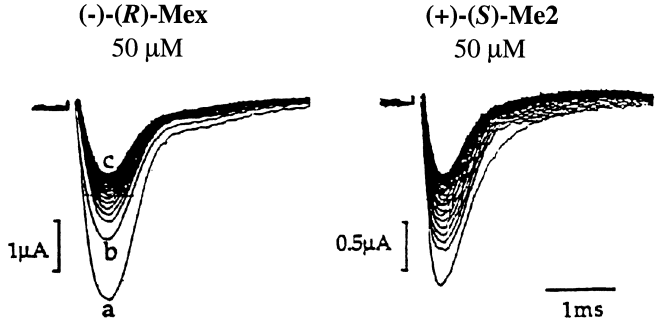

Figure 4. Inward sodium transients recorded by the three vaseline gap voltage clamp method from single frog muscle fibres and effects of (-)-(*R*)-Mex (50 μM) and (-)-(*S*)-Me2 (50 μM). After recording the control trace elicited by a single test pulse from –100 to –20 mV, the test compounds were applied (a). After 10 min of incubation, a single test pulse was again applied. The reduction of the current was the result of the tonic block by the drug (b). The application of a 30 s train of pulses at 10 Hz showed a cumulative reduction of sodium currents over the tonic block due to the use-dependent behaviour of the drug (c).

sively cumulated until a new equilibrium was reached (*figure 4*). The value of the current at this equilibrium normalized with respect to the current in the absence of the drug was used to calculate the potency of the drug in blocking the channels under excessive stimulation conditions (e.g., high-frequency firing).

In these conditions a remarkable use-dependent behaviour of Me2 was observed. In fact at 10 Hz the final block

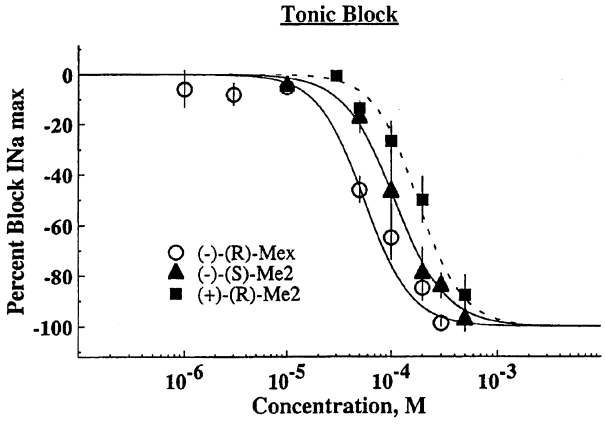

Figure 5. Concentration–response curves for the tonic block exerted by (-)-(*R*)-Mex and by both enantiomers of Me2. Each point is the mean ± SEM of the percentage block of sodium currents evaluated at each concentration from 3–5 muscle fibres.

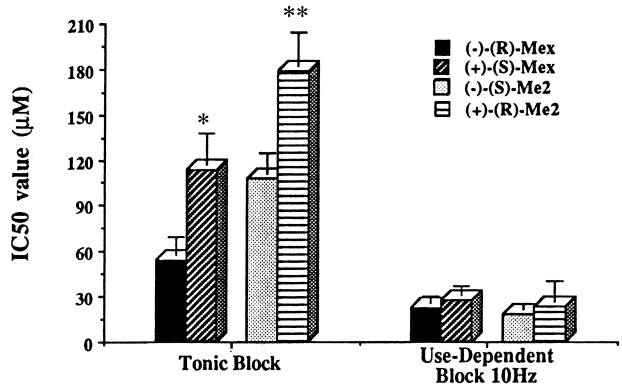

Figure 6. Each bar shows the mean ± SE of the concentrations of Mex and Me2 enantiomers able to exert a 50% reduction of sodium currents (IC_{50} values) during tonic and use-dependent block. The IC_{50} values have been obtained from the curves fitting the concentration–response experimental points [25]. At each drug concentration the use-dependent block of sodium currents has been calculated at the end of the 10 Hz stimulation, as percent reduction with respect to the current in the absence of drug. The statistical differences between fitted values have been calculated using a Student's *t* distribution using a number of degrees of freedom equal to the total number of values determining the curve, minus the number of means minus two for the free parameters. $*P < 0.01$; $**P < 0.05$.

produced by 50 μM (-)-(*S*)-Me2 was comparable to that produced by the same concentration of (-)-(*R*)-Mex (*figure 4*). Consequently, the IC_{50} value calculated for both enantiomers of Me2 was remarkably lower than that measured in tonic block (*figure 6*). The IC_{50} for (-)-(*S*)-Me2 was 6.2 times lower with respect to that of tonic block vs. the 2.4-fold lowering of IC_{50} observed for (-)-(*R*)-Mex. During the high-frequency stimulation at 10 Hz the stereoselectivity of the two compounds attenuated. In fact the eudismic ratio of Mex decreased from 2.1 to 1.2, whereas that of Me2 decreased from 1.7 to about 1 (*figure 6*) [32].

3.2. Effects of (-)-(R)-Mex and (-)-(S)-Me2 on the excitability characteristics of musle fibres of myotonic ADR mice

The myotonic ADR mouse shows a severe recessive form of myotonia due to an abnormally low resting chloride conductance caused by a genetic defect in the muscle chloride channels [25]. The myotonic hyperexcitability of sarcolemma can be clearly detected by in vitro recordings on intercostal muscle fibres. Spontaneous myotonic activity, such as high frequency discharges of action potentials (> 10 Hz) on insertion of the recording electrode, was detected in about 60–80% of fibres in each

ADR muscle preparation (*figure 7*). As typical of myotonic state, the muscle fibres of ADR mice were able to generate a significantly higher number of action potentials (11.7 ± 0.7; no. of fibres = 25) than healthy ones (5.1 ± 0.5; no. = 20) when the depolarizing stimulus was just slightly increased over the threshold values. In myotonic muscle fibres the stimulus-evoked train of action potentials was followed by self-sustained after discharges at the end of the depolarizing current pulse in about 75–100% of the fibres sampled (*figure 7*).

Both Mex and Me2, tested in the eutomeric form, reduced the hyperexcitability of myotonic muscle fibres and in particular relieved, at low concentrations, the typical myotonic manifestations (*figure 7*). (-)-(*R*)-Mex started to have a membrane-stabilizing effect at 10 μM, the concentration at which a significant reduction of the spontaneous discharges was observed. At 50 μM these discharges were completely suppressed, along with a significant reduction in the occurrence of after discharges, although the stimulus-evoked firing of the membrane was not modified (*figure 7*). The latter was appreciably reduced by 20% at 100 μM (-)-(*R*)-Mex, and about 200 μM were needed to reduce the firing capability by 50%.

(-)-(*S*)-Me2, in agreement with the strong use-dependent block of sodium currents described above, was more potent than (-)-(*R*)-Mex on the hyperexcitability of myotonic muscle fibres. In fact (-)-(*S*)-Me2 was able to decrease dose-dependently both spontaneous discharges and after discharges in the range of 5–10 μM; so (-)-(*S*)-Me2 was more potent than (-)-(*R*)-Mex on the after discharges. At the above concentrations the firing capability of the membrane was also slightly reduced (*figure 7*). (-)-(*S*)-Me2 at 50 μM completely suppressed spontaneous myotonic signs and significantly reduced the maximum number of spikes that the membrane could generate from 13 ± 0.8 (no. = 6) to 8.3 ± 0.8 (no. = 9) ($P < 0.005$), the half maximal concentration of this parameter being about 60 μM [33].

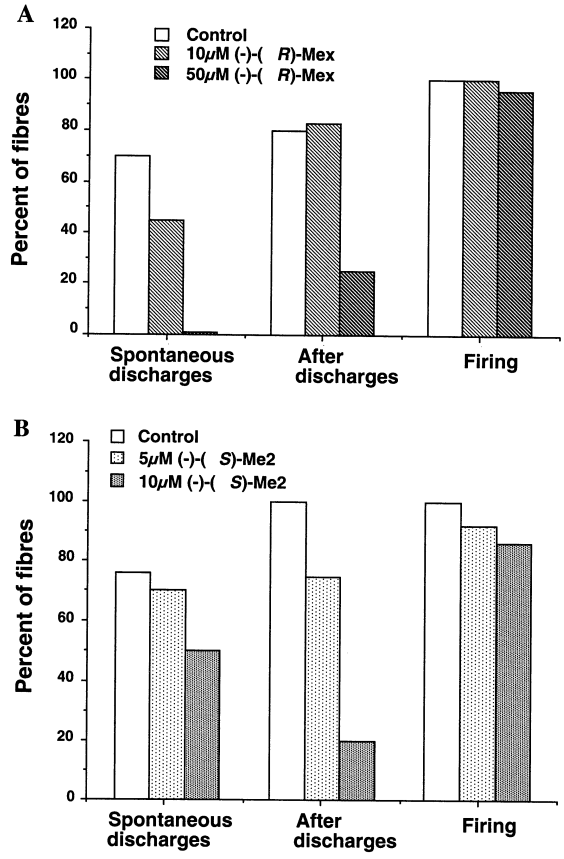

Figure 7. Effects of (-)-(*R*)-Mex and (-)-(*S*)-Me2 on the hyperexcitability of intercostal muscle fibres of myotonic ADR (arrested development of righting response phenotype) mice. In each panel the first two groups of bars from the left side show the percent of fibres sampled exhibiting spontaneous discharges, after discharges in control conditions, and further application of 10 and 50 μM of (-)-(*R*)-Mex (**A**) or 5 and 10 μM of (-)-(*S*)-Me2 (**B**). The last group of bars shows the percent reduction of the maximum number of fibres being elicited by a depolarizing stimulus in the presence of the test compounds at the above concentrations, with respect to the control taken as 100%. Statistical evaluation was based on the Chi-square test. As far as the frequency of occurrence of spontaneous discharges and of after discharges in the presence of (-)-(*R*)-Mex and (-)-(*S*)-Me2 is concerned there is a significant difference between respective groups with $\chi^2 > 6$.

5. Discussion

The use-dependent behaviour of local anaesthetic-like drugs, correlated with the affinity to and the dissociation rate from the receptor of sodium channels, is affected by variations of physical-chemical properties. A relatively high pK_a value (≥ 7.4), increasing the ratio between charged and uncharged forms at physiological pH, can improve access to the sodium channel active site by permeating the hydrophilic pathway of the open channel from the cytoplasm [19, 34], and by virtue of the voltage gradient across the membrane [35, 36]. An increase in pKa and lipophilicity values plays a role in tocainide analogues [17]. In a series of lidocaine analogues, obtained by insertion of methylene groups in the arylamide-amine chain, the affinity for the cardiac sodium channel was optimal for the next superior homologue of lidocaine. The mean IC_{50} values of lidocaine and its homologues suggested that an optimal length of the chain, per se, is important in drug binding [37]. Finally,

the increase of both molecular weight and pK_a, in another series of lidocaine and procainamide derivatives [38], as well as in a particularly high affinity series of benzocaine analogues [39], slowed the time constant governing the dissociation rate. This process, according to the 'modulated receptor' hypothesis, should take place during the membrane repolarization for the transition of the channel from the high affinity inactivated-state to the low-affinity resting state [2].

In agreement with the above-mentioned observations, the homologation of Mex with a methylene group leads to a use-dependent behaviour stronger than Mex. We hypothesize that the reduction of the electron-withdrawing effect of the xylyloxy group on the amino group, lower in Me2 than in Mex, should produce a higher charged/uncharged ratio and this could improve the access to sodium channel receptors. On the other hand, an increased lipophilicity, caused by the homologation, does favour the access to the cytoplasmic side of the membrane where the binding site is accepted to be located [18–20]; consequently, Me2 acts at lower concentrations than Mex on high-frequency discharges of action potentials of myotonic muscle fibres.

The optical isomers of Me2 bind to the receptor of sodium channels in a stereoselective manner as well as Mex [(+)-(*R*)-/(-)-(*S*)-Me2 eudismic ratio about 1.6 vs. 2 for optical isomers of Mex]; (-)-(*S*)-Me2 was less potent than (-)-(*R*)-Mex in producing tonic block but it showed a more relevant use-dependent behaviour. We recorded an attenuation of stereoselectivity when elevating the frequency of stimulation; this result might imply that the receptor on the muscle fibre sodium channel has different stereospecific requirements depending on the channel states, or that such a dissipation of stereoselectivity is due to a poor recovery from inactivation state of the drug-blocked channel during the brief interpulse duration at the 10 Hz frequency [33].

The question if there is a specific receptor for Mex and its analogues, being shared or not with α-amino xylidides, or only binding sites of a multiple nature, remains unanswered and is far from obvious. The aromatic ring of local anaesthetic-like drugs has been proposed to bind to a tyrosine residue (at position 1 586 in the rat skeletal muscle sodium channel) while the terminal amine group of the drugs may associate with a neighbouring phenylalanine residue through π electron interaction (position 1 579) [3]. Very recently, site-specific mutagenesis studies have suggested that an asparagine residue may play a role in bupivacaine binding to cardiac sodium channels [40]. Further work is needed to define the local anaesthetic-like drug receptor, if any. From a general point of view, our and others' past findings argue for a general law about primary amine LA-like drugs: the blocking property of an LA-like drug may result at least from the combination of the contribution of pK_a and LogP values. Nevertheless, other aspects, such as an optimal length of the chain, may not be discarded a priori [37]. The results here presented do parallel previous findings and confirm the bioisosteric relationship between Toc and Mex series; these observations suggest a common mechanism of action for both series. Furthermore, the Me2 eutomer, in spite of its stereochemical descriptor, does share the same configuration of the eutomers of Mex and analogues in muscle fibre sodium channel studies [41]. Thus a common binding site for Me2 and Mex may be hypothesized. This hypothesis is currently under investigation by means of eudismic analysis on newly synthesized Me2 analogues.

In conclusion, the results reported in this study demonstrate that Me2 is more potent than Mex for a use-dependent block and the candidate Me2 must undergo more complex in vivo studies which will evaluate its potential usefulness as a specific antimyotonic agent possibly overcoming Mex side effects.

5. Experimental protocols

5.1. Chemistry

Melting points were determined on a Gallenkamp melting point apparatus in open glass capillary tubes and are uncorrected. ^{1}H-NMR spectra were recorded on an FT Spectrometer Bruker Aspect 3000 (300 MHz) spectrometer using $CDCl_3$ as solvent. MS spectra were recorded with a Hewlett-Packard 5995c gas chromatograph/mass spectrometer at low resolution. Elemental analyses were performed on a Carlo Erba mod 1106 instrument, and the data for C, H, N were within ± 0.4% of theoretical values. Optical rotations were measured on a Perkin Elmer 241 MC spectropolarimeter, concentrations are expressed in g/100 mL and the cell length is 1 dm. HPLC was performed on a Waters chromatograph (Waters Assoc., Milford, MA) model 600 equipped with a U6K model injector and a 481 model variable wavelength detector. Chromatographic separations were performed on silica gel columns by flash chromatography (Kieselgel 60, 0.040–0.063 mm, Merck). TLC analyses were performed on precoated silica gel on glass or aluminium sheets (Kieselgel 60 F254, Merck). Solvents were RP grade, unless otherwise indicated. All chemicals were purchased from Aldrich in the highest quality commercially available. (±)-Mex was kindly donated by Boehringer Ingelheim KG.

*5.1.1. (-)-(S)-2-(3-hydroxy-2-methylpropyl)-1H-isoindole-1,3(2H)-dione (**2a**)*

To a stirred solution of (-)-(*R*)-3-bromo-2-methyl-1-propanol (18.9 mmol) (**1a**) in dry DMF (36 mL) under N_2 atmosphere, potassium phthalimide (6 mmol) was added and the mixture was heated to 70–80 °C. In 1 h, 6, 4 and 2 mmol of potassium phthalimide (18 mmol on the whole) were added, maintaining the temperature for 24 h. The mixture was filtered and the organic layer was concentrated in vacuo. Purification of the crude oil residue by column chromatography (petroleum ether/EtOAc 7:3) and recrystallization from CH_2Cl_2/petroleum ether gave **2a** as a white solid. Yield: 82% (72 xx). M.p. 79–80 °C. $[\alpha]^{20}_D$ = –16.0 (*c* 2, $CHCl_3$). ^{1}H-NMR: δ 0.97 (d, 3H, *J* = 7.0 Hz, CH_3–CH); 1.96–2.11 (m, 1H, CH_2–C*H*–CH_2); 2.74 (bs, 1H, CH_2O*H*); 3.34 (dd, 1H, *J* = 11.9 Hz and *J* = 6.0 Hz, CH–C*H*H–NR_2); 3.50 (dd, 1H, *J* = 11.9 Hz and *J* = 4.2 Hz, CH–CH*H*–NR_2); 3.70 (d, 2H, *J* = 6.4 Hz, OH–CH_2–CH); 7.68–7.75 (m, 2H, Ar *H*C-5, 6); 7.81–7.87 (m, 2H, Ar *H*C-4, 7) ppm. MS: m/z 219 $M^{+\cdot}$ (4),189 (46), 160 (100).

The same reaction run on **1b** gave the (+)-(*R*) enantiomer (**2b**) as a white solid. Yield and non-chiroptical physical characteristics are identical. $[\alpha]^{20}_D$ = +16.5 (*c* 2, $CHCl_3$).

*5.1.2. (+)-(S)-2-(3-bromo-2-methylpropyl)-1H-isoindole-1,3(2H)-dione (**3a**)*

Into a stirred, ice-cooled solution of **2a**, (6 mmol) in dry toluene (30 mL) under N_2 atmosphere, HBr was bubbled until saturation occurred. The mixture was stirred at –5 °C for 1 h and at room temperature for 4 h. The solvent was evaporated in vacuo, the residue was taken up with EtOAc and washed with 6 N NaOH, 2 N HCl, and H_2O. The combined organic layers were dried (Na_2SO_4) and concentrated in vacuo. Purification of the crude solid residue by recrystallization from MeOH gave **3a** as a white solid. Yield: 90% xx. M.p. 89–90 °C. $[\alpha]^{20}_D$ = +5.3 (*c* 2, $CHCl_3$). ^{1}H-NMR: δ 1.06 (d, 3H, *J* = 6.8 Hz, CH_3–CH); 2.33–2.45 (m, 1H, CH_2–C*H*–CH_2); 3.29–3.43 (m, 2H, Br–CH_2–CH); 3.59–3.75 (m, 2H, CH–CH_2–NR_2); 7.68–7.74 (m, 2H, Ar *H*C-5, 6); 7.81–7.87 (m, 2H, Ar *H*C-4, 7) ppm. MS: m/z 281 $M^{+\cdot}$ (7), 202 (21), 160 (100).

The same reaction run on **2b** gave the (-)-(*R*) enantiomer (**3b**) as a white solid. Yield and non-chiroptical physical characteristics are identical. $[\alpha]^{20}_D$ = –5.3 (*c* 2, $CHCl_3$).

*5.1.3. (+)-(S)-2-(3-iodo-2-methylpropyl)-1H-isoindole-1,3(2H)-dione (**3c**)*

Into a stirred, ice-cooled solution of **2a** (6 mmol) in dry toluene (30 mL) under N_2 atmosphere, HI was bubbled until saturation occurred. The mixture was stirred at –5 °C for 1 h and at room temperature for 4 h. The solvent was evaporated in vacuo, the residue was taken up with EtOAc and washed with 6 N NaOH, 2 N HCl, and H_2O. The organic phase was dried (Na_2SO_4) and concentrated in vacuo. Purification of the crude solid residue by column chromatography (petroleum ether/EtOAc 8:2) and recrystallization from MeOH gave **3c** as a white solid. Yield: 92% (75 xx). M.p. 83 °C. $[\alpha]^{20}_D$ = 7.2 (*c* 2, $CHCl_3$). ^{1}H-NMR: δ 1.05 (d, 3H, *J* = 6.7 Hz, CH_3–CH); 2.06–2.18 (m, 1H, CH_2–C*H*–CH_2); 3.10 (dd, 1H, *J* = 10.5 Hz and *J* = 7.5 Hz, I–C*H*H–CH); 3.23 (dd, *J* = 10.5 Hz and *J* = 6.0 Hz, 1H, I–CH*H*–CH); 3.55–3.69 (m, 2H, CH–CH_2–NR_2); 7.68–7.75 (m, 2H, Ar *H*C-5, 6); 7.81–7.88 (m, 2H, Ar *H*C-4, 7) ppm. MS: m/z 329, $M^{+\cdot}$ (< 1), 202 (77), 160 (100).

The same reaction run on **2b** gave the (-)-(*R*) enantiomer (**3d**) as a white solid. Yield and non-chiroptical physical characteristics are identical. $[\alpha]^{20}_D$ = –7.8 (*c* 2, $CHCl_3$).

*5.1.4 (+)-(S)-2-[3-(2,6-dimethylphenoxy)-2-methylpropyl]-1H-isoindole-1,3(2H)-dione (**4a**)*

To a stirred solution of 2,6-dimethylphenol (5.5 mmol) in dry DMF (30 mL) under N_2 atmosphere NaH, 60% oil dispersion (5.25 mmol), was added. After H_2 evolution ceased, **3a** (5 mmol) was added. The solution was then stirred at room temperature for 3 h then poured into a funnel separator with EtOAc and H_2O and stirred. The organic layer was washed with 2 N NaOH, and H_2O, dried (Na_2SO_4) and concentrated in vacuo. Purification of the crude oil residue by column chromatography (petroleum ether/EtOAc 8:2) and recrystallization from benzene/hexane gave **4a** as a white solid. Yield: 45% (35 xx). M.p. 73–74 °C. $[\alpha]^{20}_D$ = +5.5 (*c* 2, $CHCl_3$). ^{1}H-NMR: δ 1.09 (d, 3H, *J* = 6.9 Hz, CH_3–CH); 2.23 (s, 6H, 2CH_3–Ar); 2.51–2.62 (m, 1H, CH_2–C*H*–CH_2); 3.63–3.92 (m, 4H, O–CH_2–CH and CH–CH_2–NR_2); 6.84–6.89 (m, 1H, ArO *H*C-4′); 6.94–6.97 (m, 2H, ArO *H*C-3′, 5′); 7.67–7.72 (m, 2H, Ar *H*C-5, 6); 7.81–7.87 (m, 2H, Ar *H*C-4, 7) ppm. MS: m/z 323 $M^{+\cdot}$ (3), 160 (99), 202 (100).

The same reaction run on **3b** gave (-)-(*R*) enantiomer (**4b**) as a white solid. Yield and non-chiroptical physical characteristics are identical. $[\alpha]^{20}_D$ = –5.7 (*c* 2, $CHCl_3$).

When repeating the reaction using **3c** or **3d** as substrates, similar results were obtained.

*5.1.5. (S)-3-(2,6-dimethylphenoxy)-2-methyl-1-propanamine (***Me2***)*

To a stirred solution of **4a** (1 mmol) in absolute EtOH (10 mL), glacial AcOH (3 mmol) and $N_2H_4{\cdot}H_20$ (3 mmol) were added. The mixture was heated under reflux for 1.5 h and was filtered on a Celite pad; the solvent was evaporated in vacuo. The residue was taken up with EtOAc and extracted with 2 N HCl, then the aqueous phase was made alkaline with 2 N NaOH and extracted twice with EtOAc. The combined organic layers were dried (Na_2SO_4) and concentrated in vacuo. The final product is a colourless oil whose purity, checked by GC-MS, was considered sufficient for the subsequent reaction. MS: M/z 193 $M^{+\cdot}$ (11), 122 (54), 72 (100).

The same reaction run on **4b** gave the (*R*) enantiomer as a colourless pure oil.

*5.1.6. (-)-(S)-3-(2,6-dimethylphenoxy)-2-methyl-1-propanamine hydrochloride (***5a***)*

Into a solution of (*S*)-3-(2,6-dimethylphenoxy)-2-methyl-1-propanamine in dry Et_2O was bubbled HCl until saturation was reached. The organic phase was concentrated. Purification of the crude residue solid by recrystallization from CH_2Cl_2/petroleum ether gave **5a** as a white solid. Yield from **4a** to **5a**: 50% xx. M.p. 133–135 °C. $[\alpha]^{20}_D$ = –2.95 (*c* 2, MeOH). ^{1}H-NMR: δ 1.16 (d, 3H, *J* = 6.8 Hz, C*H*$_3$–CH); 2.24 (s, 6H, 2C*H*$_3$–Ar); 2.45–2.56 (m, 1H, CH_2–C*H*–CH_2); 3.11 (dd, 1H, *J* = 12.8 Hz and *J* = 6.8 Hz, CH–C*H*H–NH_2); 3.31 (dd, 1H, *J* = 12.8 Hz and *J* = 6.6 Hz, CH–CH*H*–NH_2); 3.64–3.75 (m, 2H, O–C*H*$_2$–CH); 6.94–6.97 (m, 3H, Ar); 8.45 (bs, 2H, CH_2–N*H*$_2$) ppm. Anal. ($C_{12}H_{20}ClNO$) C, H, N.

The same reaction run on **4b** gave the (+)-(*R*) enantiomer (**5b**) as a white solid. Yield and non-chiroptical physical characteristics are identical. $[\alpha]^{20}_D$ = +2.75 (*c* 2, $CHCl_3$). Anal. ($C_{12}H_{20}ClNO$) C, H, N.

*5.1.7. (+)-(S)-N-acetyl-3-(2,6-dimethylphenoxy)-2-methyl-1-propanamine (***6a***)*

To a stirred solution of (*S*)-Me2 (1 mmol, obtained by extraction from **5a**) in dry THF (3 mL) AC_2O (1 mmol) and Et_3N (1 mmol) were added and the solution was stirred at room temperature for 8 h. The solvent was evaporated in vacuo and the residue was taken up with EtOAc and washed with a saturated aqueous solution of $NaHCO_3$ and brine. The organic layer was dried (Na_2SO_4) and concentrated in vacuo. Yield from **5a** to **6a**: 52%. M.p. 84–86 °C. $[\alpha]^{20}_D$ = +6.6 (*c* 2, $CHCl_3$). ^{1}H-NMR: δ 1.05 (d, 3H, *J* = 7.0 Hz, C*H*$_3$–CH); 1.98 (s, 2H, 2C*H*$_3$–CO); 2.14–2.22 (m overlap s at 2.26, 1H, CH_2–C*H*–CH_2); 2.26 (s, 6H, 2C*H*$_3$–Ar); 3.27–3.36 (m, 1H, CH–C*H*H–NHR); 3.46–3.54 (m, 1H, CH–CH*H*–NHR); 3.63–3.73 (m, 2H, O–C*H*$_2$–CH); 6.24 (bs, 1H, CH_2–*NH*–CO); 6.89–6.92 (m, 1H, Ar *H*C-4); 7.00 (apparent d, 2H, Ar *H*C-3, 5) ppm. MS: m/z 235 $M^{+\cdot}$ (< 1), 121 (15), 114 (100).

The same reaction run on (*R*)-Me2 gave the (*R*) enantiomer (**6b**). Yield and non-chiroptical physical characteristics are identical. $[\alpha]^{20}_D$ = –6.4 (*c* 2, $CHCl_3$).

5.2. Pharmacology

5.2.1. Recordings of sodium current using the three vaseline gap voltage clamp technique

Voltage clamp recordings of sodium currents were performed on 1–2 cm long segments of single muscle fibres obtained by microsurgery from the ventral branch of the semitendinosus muscle of *Rana esculenta*. The cut end fibre was superfused with an internal solution of 105 mM CsF, 5 mM MOPS, 2 mM $MgSO_4$, 5 mM EGTA and 0.55 mM Na_2ATP (pH = 7.2 with NaOH concentrated solution) and transferred into the recording chamber, filled with the same solution, as described in detail elsewhere [14, 42]. Four KCl/agar bridge electrodes connected each of the four pools of the recording chamber to the voltage clamp amplifier [14]. Then the solution in one of the central pools (70–100 μm wide) was replaced by an external solution of 77 mM NaCl, 38 mM Coline-Cl, 1.8 mM $CaCl_2$, 2.15 mM Na_2HPO_4 and 0.85 mM NaH_2PO_4. Recordings were performed at 10 °C. The circuit of the voltage clamp amplifier was based on that described by Hille and Campbell [42]. The holding potential (h.p.) was –100 mV. The voltage clamp amplifier was connected via a 12-bit AD/DA interface (Digidata 1200, Axon instruments, Forster city, CA, USA) to an 80486 DX2/66 PC. The stimulation protocols and data acquisitions were driven by the Clampex program (pClamp 6 software package, Axon Instruments). The sodium currents flowing in response to depolarizing command voltages were lowpass filtered at 10 kHz (Frequency Devices, USA) visualized on an oscilloscope, sampled at 20 kHz and stored on the hard disk. When necessary leak and capacity currents were subtracted using the P/4 method. The acquired traces were later analysed with the Clampfit program (pClamp 6 software package Axon Instruments). Maximal sodium currents ($I_{Na\ max}$) were elicited with test pulses from the h.p. to –20 mV for 10 ms. The tonic block exerted by the test compounds was evaluated as percent reduction of the peak sodium current elicited by single test pulses. The evaluation of the use-dependent block by the drugs was made by using a 10 Hz train of test pulses for a period of 30 s and by normalizing the residual current at the end of

this stimulation protocol with respect to that in the absence of the drug [14].

5.2.2. Current clamp recordings of macroscopic membrane excitability of ADR myotonic mice

Myotonic ADR (genotype *adr/adr)* mice of 3 months were used for all the experiments [25, 26]. The intercostal muscle was dissected under urethane anaesthesia (1.2 g/kg i.p.), and immediately placed in a muscle bath containing a normal physiological solution of 148 mM NaCl, 4.5 mM KCl, 2.0 mM $CaCl_2$, 10 mM $MgCl_2$, 12 mM $NaHCO_3$, 0.44 mM NaH_2PO_4 and 5.55 mM glucose. The perfusing solution was continuously gassed with 95% O_2 and 5% CO_2 (pH = 7.2–7.4) and the electrophysiological recordings were performed at 30 °C. The excitability parameters of sarcolemma were recorded with the two intracellular microelectrode technique [14, 43]. A voltage sensing electrode and a current passing electrode were placed 50 μm apart in the central region of randomly selected superficial fibres. After the recording of the membrane resting potential (RMP), the fibres were current-clamped at –80 mV. The excitability characteristics of the sampled fibres were determined by recording the intracellular membrane potential response to square-wave depolarizing constant current pulses. The current intensity was gradually increased until the depolarization was just sufficient to elicit a single action potential. The current intensity was then further increased to generate two or more action potentials. In this way it was possible to record and measure the various excitability parameters [14]. The antimyotonic activity of the test compounds has been evaluated at the maximum number of action potentials that the muscle fibres could generate (N spikes) which is abnormally increased in myotonic muscle [26, 44]. Other peculiar features of the myotonic fibres were the occurrence of spontaneous discharges (SpD) of action potentials on the insertion of the voltage sensing microelectrode and of self sustained after discharges (AD), i.e. discharges of action potential after the end of the application of the depolarizing stimulus [26, 44]. Thus, in each preparation, the number of fibres showing either spontaneous discharges or after discharges was calculated as a percentage of the total number of fibres sampled both in the absence and in the presence of the drugs in order to evaluate their ability to suppress these pathological manifestations in each individual muscle preparation.

5.2.3. Drugs

Stock solutions of each enantiomer of Mex and Me2 were prepared daily in physiological or external solutions for current clamp and voltage clamp experiments. The final concentrations to be tested in vitro were obtained by further diluting the stock solution as needed. On both mouse intercostal muscle and frog muscle fibres, no more than three concentrations of the same compound were tested, and the preparations were exposed to each concentration for at least 10 min before recording to allow the maximum effect of the drug to be reached.

5.2.4. Statistical analysis

The data were expressed as mean ± SEM. The statistical significance of the differences between groups of means was calculated by unpaired Students *t*-test. The molar concentrations of the drugs producing a 50% block of $I_{Na\ max}$ (IC_{50}) were determined by using a non-linear least square fit of the concentration–response curves to the logistic equation described in detail elsewhere [14]. The estimates of SE and *n* for normalized percent values were obtained as described by Green and Margerison [45].

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