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Synthesis and biological evaluation of *N*-methyl-laudanosine iodide analogues as potential SK channel blockers

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Abstract—Neuronal action potentials are followed by an afterhyperpolarisation (AHP), which is mediated by small conductance Ca^{2+}-activated K^+ channels (SK channels or KCa2 channels). This AHP plays an important role in regulating neuronal activity and agents modulating AHP amplitude could have a potential therapeutic interest. It was previously shown that *N*-methyl-bicuculline iodide blocks SK channels but its $GABA_A$ activity represents a serious drawback. In view of the structural analogy between bicuculline and laudanosine **14**, several N-quaternary analogues of the latter were developed. It was shown that *N*-methyl-laudanosine **15** (NML) and *N*-ethyl-laudanosine **16** induce a reversible and relatively specific blockade of the apamin sensitive AHP in dopaminergic neurones with mean IC_{50}s of 15, and 47 μM, respectively. Laudanosine **14**, *N*-butyl-**17** and *N*-benzyl-**18** derivatives were less potent. In order to find pharmacophore elements, modifications were performed at different positions such as C-1, C-6 and C-7. Intracellular recordings on rat midbrain dopaminergic neurones were made in order to evaluate the putative blockade of SK channels by these molecules. Simplified structures such as tetrahydroisoquinoline derivatives with H or Me at C-1 **1**–**6** presented no significant activity at 300 μM. The presence of a 1-(3,4-dimethoxybenzyl) moiety seems an important feature. Indeed, compound **8** showed a blockade of the AHP of only 33% at 300 μM while compound **13** blocked it by 67%, respectively, at the same concentration. Binding experiments were also performed. Binding affinities for SK channels are in good agreement with electrophysiological data. These results indicate that the presence of a charged nitrogen group is an essential point for the affinity on SK channels. Finally, because of the similar activity of both enantiomers of NML **19** and **20**, the interaction site may present a symmetrical configuration.

1. Introduction

Three families of calcium-activated potassium channels have been identified, which can be separated on the basis of their biophysical and pharmacological properties. So the three families are called BK, IK and SK reflecting their large, intermediate and low conductance, respectively.[1,2]

Small conductance Ca^{2+}-activated K^+ (SK) channels underlie the prolonged postspike afterhyperpolarisation (AHP), which plays an important role in modulating the firing rate and the firing pattern of neurones.[3,4] Three members of the SK family have been cloned: SK1, SK2 and SK3.[3] In contrast to BK channels, SK channels are voltage insensitive and are not blocked by low concentrations of tetraethylammonium[5] or charybdotoxin,[6] but are only activated by an increased intracellular calcium concentration. The distribution of the SK channel subtypes was investigated in the rat by using in situ hybridization and immunohistochemistry and revealed that SK1 and SK2 subtypes are mostly expressed in the cortex and hippocampus[7] while SK3 channels expression is higher in subcortical areas, especially in the monoamine cell regions, for example, substantia nigra, dorsal raphe and locus coeruleus. These characteristics attract considerable attention to SK channels as putative targets for indications in cognitive dysfunction,[8–12] neuronal hyperexcitability,[13] dopamine-related disorders,[14–16] and depression.[11]

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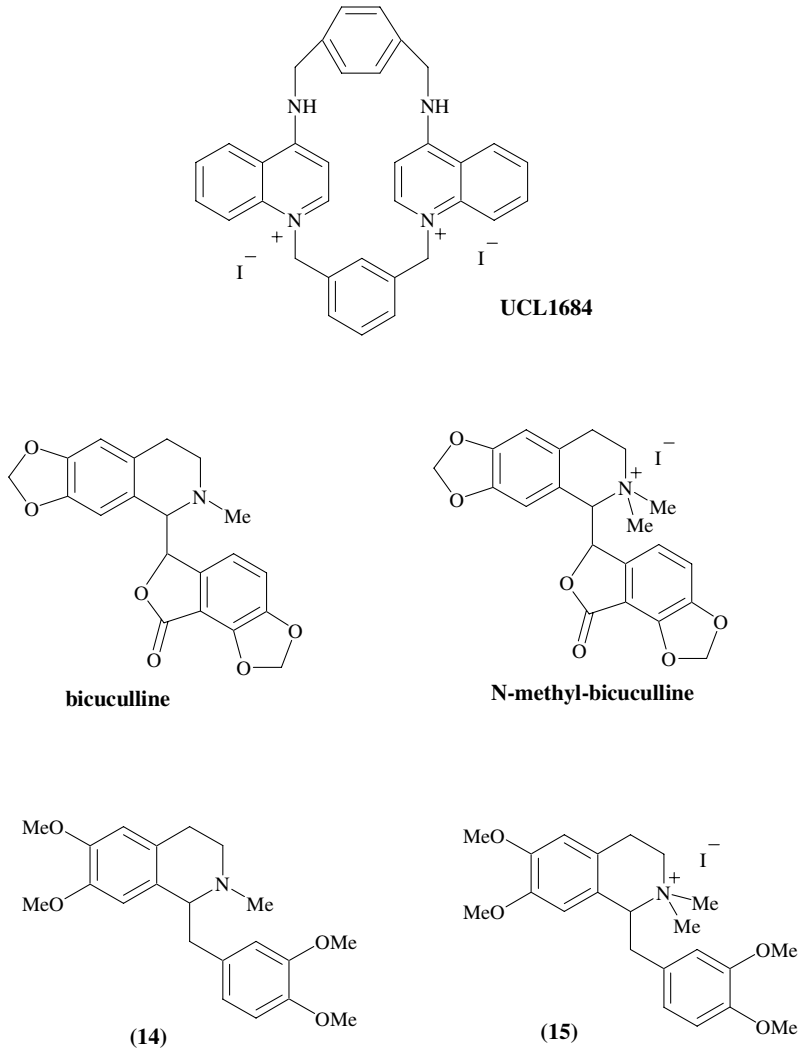
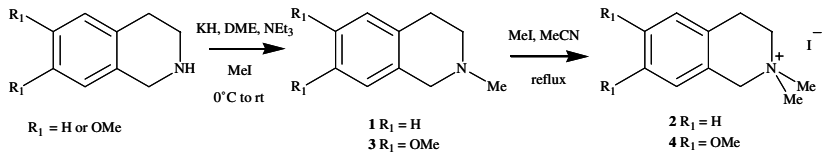


Figure 1. Chemical structures of UCL 1684, bicuculline, *N*-methyl-bicuculline, laudanosine **14** and *N*-methyl-laudanosine iodide **15**.

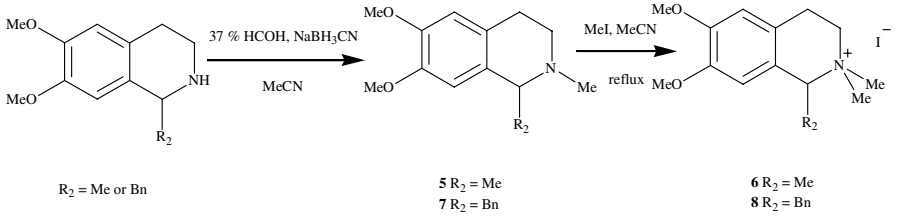
So far, the most potent SK channel blockers are venom toxins such as apamin and leiurotoxin I. Apamin is an octadecapeptide isolated from honey bee *Apis mellifera* venom. This peptide presents two arginine residues in contiguous positions and a rigid cyclic conformation due to two disulfide bridges.[17] Leiurotoxin I isolated from scorpion *Androctonus mauretanicus mauretanicus* potently blocks human SK2 and SK3 but not SK1 channels.[18] Dequalinium, a non-peptidic ligand, presents some SK channel blocking properties and extensive structure–activity relationship studies led to UCL 1684 (Fig. 1).[19] On the other hand, *N*-methyl-bicuculline (Fig. 1) was reported to potentiate burst firing in dopaminergic neurones by blocking the apamin-sensitive Ca^{2+}-activated K^+ current.[20] Because of the $GABA_A$ antagonist activity of bicuculline quaternary salts, more selective compounds were needed. Therefore we decided to develop new SK blockers based on a SAR of the bicuculline structure. First studies started with laudanosine **14** (Fig. 1), an alkaloid having a structure quite related to *N*-methyl-bicuculline. From the *N*-methyl quaternary analogue, several structural modifications were made in order to find some pharmacophore elements and the corresponding molecules were tested for their effect on the apamin-sensitive AHP of dopaminergic neurones. Their binding affinities for SK channels were also evaluated.

2. Chemistry

N-methylation of 1,2,3,4-tetrahydroisoquinoline derivatives was performed by two methods depending on the chemical series. In the first procedure (Scheme 1), 1,2,3,4-tetrahydroisoquinoline derivatives were converted to the corresponding potassium amide by reaction with potassium hydride and triethylamine in 1,2-dimethoxyethane (DME) at 0 °C and after addition of methyl iodide the tertiary amines **1** and **3** were isolated. Finally the *N*-methyl derivatives **2** and **4** were prepared by treatment with methyl iodide.[21] In the second case, 1-substituted tetrahydroisoquinoline analogues were methylated by reductive alkylation using sodium cyanoborohydride and paraformaldehyde to give the tertiary amines **5** and **7** (Scheme 2).[22] The *N*-methyl derivatives **6** and **8** were prepared by treatment with methyl iodide.[21]



Scheme 1. Preparation of 2,2-dimethyl-1,2,3,4-tetrahydroisoquinolinium analogues.



Scheme 2. Preparation of 1-substituted-2,2-dimethyl-1,2,3,4-tetrahydroisoquinolinium derivatives.

Compound **10** was synthesized by benzylation and hydrolysis of the Reissert compound **9** obtained by reaction of potassium cyanide and benzoyl chloride with isoquinoline in a mixture of methylene chloride and water (Scheme 3).[23] Compound **10** was further methylated by methyl iodide in refluxing MeCN. The resulting isoquinolinium iodide **11** was then reduced by using sodium borohydride in methanol to afford the methylated amine **12**.

Quaternary ammonium iodides (**2**, **4**, **6**, **8**, **13**) were finally obtained by treatment of the *N*-methyl-1,2,3,4-tetrahydroisoquinoline derivatives (**1**, **3**, **5**, **7**, **12**) with methyl iodide in MeCN (Scheme 3).

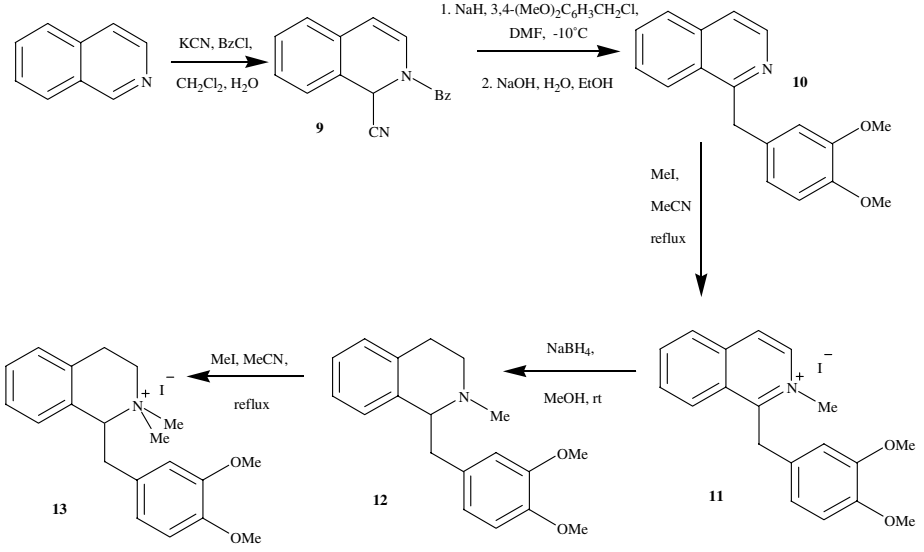
N-alkyl or benzyl derivatives of laudanosine **15**–**18** were synthesized from (+/−) laudanosine **14** in the presence of the corresponding alkyl iodide or benzyl chloride.

Otherwise, from (±)-laudanosine **14** both enantiomers were separated by chiral preparative HPLC and further methylated by an excess of methyl iodide in refluxing acetonitrile to give the (+)- and (−)-enantiomers **19** and **20**.

3. Results

The biological data are summarized in Table 1. None of the simplified structures such as tetrahydroisoquinoline derivatives with a hydrogen in position 1 **1**–**4** and compound **5** showed any significant activity on the AHP at concentrations up to 300 μM but compounds **6** and **12** presented a very slight blockade of 22.7% ± 4.6% ($n = 3$) and 22.4% ± 1.8% ($n = 3$) of the AHP at 300 μM, respectively. In the 1-benzyl series, the tertiary amine **7** induced a slight blockade of the AHP of 47% while the quaternary derivatives compounds **8** and **13** induced a blockade of the apamin-sensitive AHP of 33.3% ± 9.5% ($n = 3$) and 67.3% ± 9.3% ($n = 3$) at 300 μM, respectively (Fig. 2).

The enantiomers of *N*-methyl-laudanosine blocked the AHP with an IC_{50} of 17.3 μM ± 2.3 μM ($n = 3$) and



Scheme 3. Synthesis of 1-(3,4-dimethoxybenzyl)-2,2-dimethyl-1,2,3,4-tetrahydroisoquinolinium iodide **13**.

Table 1. Effects of *N*-methyl laudanosine and analogues on the apamin-sensitive AHP of DA neurones and binding affinities for cortical apamin-sensitive sites

Compounds	Blocking potential of the AHP in dopaminergic neurones (IC_{50} ± SEM, μM)	In vitro binding on SK channels	
		(IC_{50} ± SD μM)	% of ^{125}I-apamin displaced[d]
(**1**)	NA[a]	—	—
(**2**)	NA[a]	—	—
(**3**)	NA[a]	—	—
(**4**)	NA[a]	—	—
(**5**)	NA[a]	—	—
(**6**)	22.6 ± 6.5%[b]	—	17%
(**7**)	47.0% ± 6.2%[b]	—	21%
(**8**)	33.3% ± 9.5%[b]	—	10%
(**12**)	22.4% ± 1.8%[b]	—	10%
(**13**)	67.3% ± 9.7%[b]	—	25%
Laudanosine (**14**)	152 ± 8	40.2[c]	10%
N-methyl-laudanosine (**15**)	15 ± 1	8.7 ± 2.3 (3.9[c])	57%
N-ethyl-laudanosine (**16**)	26.4 ± 7.5	47.8[c]	—
N-butyl-laudanosine (**17**)	17.3 ± 2.3	>250[c]	—
N-benzyl-laudanosine (**18**)	47 ± 6	56.4[c]	—
(+)-*N*-methyl-laudanosine (**19**)	>300	7.6 ± 0.9	57%
(−)-*N*-methyl-laudanosine (**20**)	249 ± 39	7.6 ± 1.2	54%
Apamin	—	20.4 ± 5.7 pM	—

[a] NA means no activity at 300 μM.
[b] % of blockade of the AHP in dopaminergic neurones at 300 μM.
[c] After Scuvée-Moreau et al. 2002.[24]
[d] % of ^{125}I-apamin displaced at 10 μM.

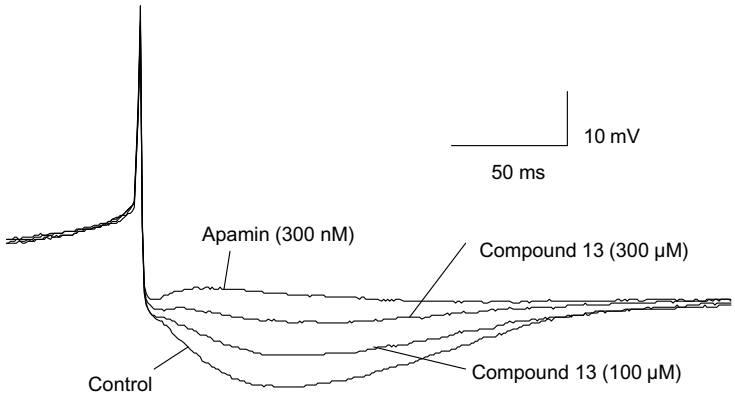


Figure 2. Effect of increasing concentrations of compound **13** on the AHP of midbrain dopaminergic neurons. While 100% blockade of the AHP is obtained with 300 nM apamin, 67.3% blockade is obtained with 300 μM compound **13**. Each trace is the mean of four sweeps. Action potentials are truncated.

26.4 μM ± 7.5 μM (n = 3), respectively, for the (+) and (−) isomers.

In binding experiments, the affinity of NML **15** (IC_{50} = 8.7 μM) and of apamin (IC_{50} = 20.4 pM) were similar to those reported in a previous publication.[24] Binding results did not show any significant difference in affinity of both enantiomers **19** and **20** (IC_{50} = 7.6 μM) for apamin binding sites. Among recently prepared derivatives only compounds **7** and **13** displaced more than 20% of ^{125}I-apamin at 10 μM.

4. Discussion

Firstly it was demonstrated that *N*-methyl-bicuculline was an interesting blocker of the apamin-sensitive AHP in dopaminergic neurones with a poor selectivity.[25] Then *N*-methyl-laudanosine **15** was synthesized and tested for its blockade of apamin-sensitive AHP. This compound **15** revealed an interesting binding profile and a very quickly reversible effect on the apamin-sensitive AHP.[24] We therefore decided to synthesise and evaluate enantiomers of *N*-methyl-laudanosine and compounds structurally close to *N*-methyl-laudanosine template.

Apamin has two arginine residues and it was demonstrated that these guanidinium functions are necessary for the activity of this peptide.[18,26,27] All other peptides that block SK channels contain at least one charged residue. This fact is also a common characteristic of non-peptidic ligands such as dequalinium and its derivatives such as UCL 1684 or with neuromuscular blockers

(atracurium, pancuronium and tubocurarine),[28,29] which also block the AHP. From these studies, it was also clearly shown that the quaternization of the amine in this series seems a crucial point to produce an activity on the AHP mediated by SK channels.

We show here that a benzyl substituent at the C-1 position (**8**, **13**) is necessary for the activity of the compounds while a smaller group (**2**,**4**,**6**) is unfavourable to block the AHP of dopaminergic neurones. Moreover, the 3,4-dimethoxybenzyl substituent (**13**) appears more effective than an unsubstituted benzyl group (**8**) for the blockade of the AHP. This observation suggests that a bulky group or a H bond acceptor could be important for interactions within the channel but more examples are necessary to confirm this hypothesis.

By modulating the group on the nitrogen of laudanosine it was previously shown[24] that a methyl moiety (**15**) presents the best blocking potency. Ethyl (**16**), butyl (**17**) and benzyl (**18**) groups induce a clear decrease of the activity. Binding experiments on SK channels gave the following IC_{50s}: 3.9 μM for *N*-methyl-(**15**), 47.8 μM for N-ethyl-(**16**), >250 μM for *N*-butyl-(**17**) and 56.4 μM for *N*-benzyl-(**18**) analogues.[24] The tertiary amine, laudanosine **14** was also tested and showed only a weak affinity in binding experiments (IC_{50} of 40.2 μM). These results are in agreement with the experiments performed on rat brain slices and confirm the micromolar affinity of *N*-methyl-laudanosine on SK channels. In addition it was shown that *N*-methyl-laudanosine has no affinity for voltage-sensitive potassium channels and α-bungarotoxin-insensitive nicotinic receptors, a weak affinity for muscarinic receptors (IC_{50} = 114 μM) and a low affinity for α-bungarotoxin-sensitive nicotinic receptors (IC_{50} = 367 μM).[24]

The interest to find a selective blocker of the SK channels with an IC_{50} in the micromolar range is to have a highly reversible ligand that permits to explore the pharmacology of these channels more easily in comparison with apamin. Indeed, apamin is destroyed by protease due to this peptidic nature[30] and its interaction with SK channels is very slowly reversible, making it difficult to observe a wash-out of the drug (Scuvée-Moreau, personal observation). Another advantage of these ammonium iodides is their high solubility in water and also the permanent charge on the nitrogen, both parameters being interesting for iontophoresis experiments.[31]

Moreover it was shown that, unlike *N*-methyl-bicuculline, *N*-methyl-laudanosine iodide has no antagonistic effect on $GABA_A$ receptors.[24]

N-methyl-laudanosine **15** being a racemic mixture, both enantiomers **19** and **20** were isolated from laudanosine **14** by chiral preparative HPLC, and after methylation, these isomers were tested in electrophysiological and in binding experiments. No clear difference was found between both enantiomers in binding studies and both isomers appeared quite equivalent for blocking the apamin-sensitive AHP in dopaminergic neurones. Thus these results suggest the presence of one symmetry element in the binding site.

5. Conclusion

Different compounds structurally related to laudanosine were prepared and tested in order to find the structural elements important for the activity on the apamin-sensitive sites. These results show that both enantiomers of NML have the same affinities for the apamin binding sites and a quite similar effect on the AHP of dopaminergic neurons. Moreover quaternization of the nitrogen in position 2 appears to be an important parameter for affinities and activities on SK channels. Our data thus suggest that the pharmacophore should present some symmetrical environment. Finally, the 3,4-dimethoxybenzyl moiety seems to be crucial for affinities on apamin binding sites in 1-substituted-1,2,3,4-tetrahydroisoquinoline series. Further chemical and biological studies are in progress for examining other structural elements of NML or related drugs.

6. Experimental sections

6.1. Chemistry

Melting points were determined on a Büchi-Tottoli capillary melting point apparatus in open capillary and are uncorrected. NMR spectra were recorded on a Bruker AM400 spectrometer at 400 MHz with external standard. IR spectra were obtained on a Perkin–Elmer FTIR-1750 spectrometer using KBr discs and only significant bands from IR were reported. Elemental analyses were determined using a Carlo-Erba elemental analyser CHNS-O model EA 1108 and were within 0.4% of theoretical values. Mass spectra were recorded on a QTOF II (micromass, Manchester UK) spectrometer with electrospray mode. Optical activity was determined on an optical activity AA-10 polarimeter at room temperature. Separations by flash chromatography were carried out using Merck Kieselgel 60 (230–400 mesh). All starting materials and reagents were obtained from Aldrich Chemical Co. or Acros chemicals and were used without further purification. Concentration and evaporation refer to removal of volatile materials under reduced pressure (10–15 mmHg at 30–50 °C) on a Buchi Rotavapor.

6.2. 2-Methyl-1,2,3,4-tetrahydroisoquinoline fumarate (1)

1,2,3,4-Tetrahydroisoquinoline (5 g; 37.5 mmol) was dissolved in 1,2-dimethoxyethane (30 mL). Triethylamine (5,2 mL; 375 mmol) and KH (7.5 g; 56.25 mmol) were added under N_2 at 0 °C. After 30 min methyl iodide (3.5 mL; 56.25 mmol) was added and the mixture was stirred overnight at room temperature. The mixture was extracted with CH_2Cl_2 (3 × 75 mL) after the addition of a saturated aqueous NH_4Cl (30 mL) and a 10% aqueous NaOH to basify. The organic solution was dried on anhydrous $MgSO_4$ and evaporated under reduced pressure to give an oil, which was purified by flash chromatography (eluent CH_2Cl_2/MeOH 97:3). The

resulting oil was crystallized as fumarate salt from EtOH (1.1 g; 11%). mp: 149–151 °C. ^{1}H NMR (DMSO-d_6) δ 7.16–7.14 (m, 4H, Ar*H*); 6.57 (s, 2H, fumarate); 3.73 (s, 2H, phenyl–CH_2–N); 2.89–2.84 (dd, 4H, CH_2–CH_2–N); 2.51 (s, 3H, CH_3). IR (KBr, cm^{-1}) 2670, 2597, 1705. Anal. $C_{14}H_{17}NO_4$ (263.293) calcd: N, 5.32; C, 63.86; H, 6.51. Found: N, 5.41; C, 63.82; H, 6.79.

6.3. 2,2-Dimethyl-1,2,3,4-tetrahydroisoquinolinium iodide (2)

2-Methyl-1,2,3,4-tetrahydroisoquinoline fumarate (0.50 g; 1.89 mmol) was solubilized in water (20 mL) and then basified with 10% aqueous NaOH. The base was extracted with CH_2Cl_2 (3 × 25 mL). The organic layer was dried and evaporated under reduced pressure to give an oil, which was dissolved in MeCN. Methyl iodide (1.17 mL; 18.9 mmol) was added and the mixture was refluxed overnight. Solvent was eliminated under reduced pressure and the crude residue was crystallized from MeOH/Et_2O (0.43 g; 86%). mp: 188–189 °C. ^{1}H NMR (DMSO-d_6): δ 7.36–7.17 (dd, 4H, Ar*H*); 4.61 (s, 2H, phenyl–CH_2–N); 3.70 (t, 2H, phenyl–CH_2–CH_2–N); 3.33 (t, 2H, phenyl–CH_2–CH_2–N); 3.15 (s, 6H, 2 × NCH_3). IR (KBr, cm^{-1}) 3001, 1478, 1460, 1436, 762. Anal. $C_{11}H_{16}NI$ (289.156) calcd: N, 4.84; C, 45.69; H, 5.58, Found: N, 4.97; C, 45.47; H, 5.63.

6.4. 6,7-Dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline fumarate (3)

This compound was obtained from 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline using the method described for compound **1**. The product was crystallized from EtOH as fumarate salt (39%). mp: 175–177 °C. ^{1}H NMR (DMSO-d_6) δ 6.69 (s, 1H, Ar*H*); 6.64 (s, 1H, Ar*H*); 6.52 (s, 2H, fumarate); 3.69 (s, 8H, 2 × *OCH*$_3$ et Ar–CH_2–N); 2.87–2.8 (dd, 4H, CH_2–CH_2–N); 2.49 (s, 3H, NCH_3). IR (KBr, cm^{-1}) 2598, 1702. Anal. $C_{16}H_{21}NO_6$ (323.341) calcd: N, 4.33; C, 59.43; H, 6.55. Found: N, 4.65; C, 59.38; H, 6.74.

6.5. 6,7-Dimethoxy-2,2-dimethyl-1,2,3,4-tetrahydroisoquinolinium iodide (4)

Compound **4** was prepared from 6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline fumarate using the method described for compound **1** but in the presence of a large excess of methyl iodide (10 equiv). The product was crystallized from MeOH/Et_2O (25%). mp: 234–236 °C. ^{1}H NMR (DMSO-d_6) δ 6.88 (s, 1H, Ar*H*); 6.75 (s, 1H, Ar*H*); 4.48 (s, 2H, phenyl–CH_2–N); 3.73 (d, 6H, 2 × OCH_3); 3.6 (t, 2H, phenyl–CH_2–CH_2–N); 3.1 (s, 6H, 2 × NCH_3); 3.0 (s, 2H, phenyl–CH_2–CH_2–N). IR (KBr, cm^{-1}) 2968, 1519, 1116. Anal. $C_{13}H_{20}NO_2I$ (349.208) calcd: N, 4.01; C, 44.71; H, 5.77. Found: N, 4.05; C, 44.87; H, 5.93.

6.6. 6,7-Dimethoxy-1,2-dimethyl-1,2,3,4-tetrahydroisoquinoline hydrochloride (5)

6,7-Dimethoxy-1-methyl-1,2,3,4-tetrahydroisoquinoline (0.41 g; 2 mmol) was dissolved in MeCN (20 mL) followed by a 37% aqueous formaldehyde (1.84 mL; 24 mmol) and $NaBH_3CN$ (0.15 g; 2.4 mmol). After 30 min, acetic acid was added until neutral and the mixture was stirred overnight. The solvent was removed under reduced pressure and the crude residue was dissolved in a 2N aqueous NaOH (15 mL). This suspension was extracted with $CHCl_3$ (3 × 15 mL). The organic layer was washed with brine and dried with anhydrous $MgSO_4$. The solvent was evaporated under reduced pressure to gave an oil. The compound was isolated as a hydrochloride salt and recrystallized from MeCN/Et_2O (0.338 g; 71%). mp: 204–205 °C. ^{1}H NMR ($CDCl_3$) δ 6.62 (s, 1H, Ar*H)*; 6.52 (s, 1H, Ar*H*); 4.25 (d, 1H, phenyl–C*H*–N); 3.86 (s, 6H, 2 × OCH_3); 3.36–2.77 (m, 4H, phenyl–CH_2–CH_2–N); 1.96 (s, 3H, NCH_3); 1.86–1.83 (dd, 3H, CH_3). IR (KBr, cm^{-1}) 2458, 1522. Anal. $C_{13}H_{20}NO_2Cl$ (257.758) calcd: N, 5.43; C, 60.58; H, 7.82. Found: N, 6.08; C, 60.56; H, 8.03.

6.7. 6,7-Dimethoxy-1,2,2-trimethyl-1,2,3,4-tetrahydroisoquinolinium iodide (6)

A solution of 6,7-dimethoxy-1,2-dimethyl-1,2,3,4-tetrahydroisoquinoline (0.44 g; 2 mmol) in MeCN (20 mL) was stirred with anhydrous K_2CO_3 (5.6 g; 40 mmol) during 30 min. Methyl iodide (0.19 mL; 40 mmol) was added. After 1 h the precipitate was filtered and the resulting solution evaporated under reduced pressure. Finally the residue was crystallized from EtOH (0.224 g; 30%). mp: 203–204 °C. ^{1}H NMR (DMSO-d_6) δ 6.83 (s, 1H, Ar*H*); 6.79 (s, 1H, Ar*H*); 4.62 (s, 1H, phenyl–C*H*–N); 3.74 (s, 6H, 2 × OCH_3); 3.58 (m, 2H, phenyl–CH_2–*CH*$_2$–N); 3.33 (d, 2H, phenyl–CH_2–CH_2–N); 3.11–3.05 (d, 6H, 2 × NCH_3); 1.6 (d, 3H, CH_3). IR (KBr, cm^{-1}) 2990, 1520. Anal. $C_{14}H_{22}NO_2I$ (363.235) calcd: N, 3.86; C, 46.29; H, 6.10. Found: N, 3.92; C, 45.90; H, 6.20.

6.8. 1-Benzyl-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline hydrochloride (7)

Compound **7** was obtained from 1-benzyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline using the method described for compound **5**. Finally the residue was crystallized from EtOH/Et_2O (53%). mp: 184–186 °C. ^{1}H NMR ($CDCl_3$) δ 6.98 (s, 5H, Ar*H*); 6.52 (s, 2H, Ar*H*); 5.43 (s, 1H, benzyl–C*H*–N); 3.74 (s, 6H, 2 × OCH_3); 3.29 (s, 2H, phenyl–CH_2–CH_2–N); 3.24 (s, 2H, C_6H_5–CH_2–); 2.98–2.88 (d, 2H, phenyl–CH_2–CH_2–N); 2.79 (d, 3H, NCH_3). IR (*KBr*, cm^{-1}) 2434, 1520, 1266. Anal. $C_{19}H_{24}NO_2Cl$ (333.859) calcd: N, 4.19; C, 68.35; H, 7.25. Found: N, 4.60; C, 68.07; H, 7.88.

6.9. 1-Benzyl-6,7-dimethoxy-2,2-dimethyl-1,2,3,4-tetrahydroisoquinolinium iodide (8)

Compound **8** was prepared from compound **7** using the method described for compound **6**. Finally the residue was crystallized from EtOH/Et_2O (18%). mp: 146–147 °C. ^{1}H NMR (DMSO-d_6) δ 7.66–7.64 (d, 2H, Ar*H*); 7.40–7.26 (m, 5H, Ar*H*); 6.9 (s, 1H, benzyl–C*H*–N); 3.8 (s, 2H, C_6H_5–CH_2–); 3.42 (s, 2H, phenyl–

CH_2–CH_2–N); 3.35 (s, 6H, $2 \times OCH_3$); 3.19 (s, 6H, $N(CH_3)_2$); 3.11 (s, 2H, phenyl–CH_2–CH_2–N). IR (KBr, cm^{-1}) 2985, 1517. Anal. $C_{20}H_{26}NO_2I$ (439.339) calcd: N, 3.19; C, 54.68; H, 5.97. Found: N, 3.25; C, 54.80; H, 5.94.

6.10. 2-Benzoyl-1-cyano-1,2-dihydroisoquinoline (9)

This product was obtained by using a method previously described.[23] The compound was crystallized from EtOH (47.04 g; 72%). mp: 124–125 °C. ^1H NMR ($CDCl_3$) δ 7.0–7.7 (m, 9H, Ar*H*); 6.60 (s, 1H, H_3); 6.55 (s, 1H, H_1); 6.05 (d, 1H, H_4). IR (KBr, cm^{-1}) 2239, 1660, 1632. Anal. $C_{17}H_{12}N_2O$ (260.297) calcd: N, 10.76; C, 78.44; H, 4.65. Found: N, 10.86; C, 78.21; H, 4.94.

6.11. 1-(3,4-Dimethoxybenzyl)-isoquinoline (10)

Compound **10** was prepared as previously described.[23] The crude residue was recrystallized in petroleum ether 100–140 °C. (84%). mp: 70–72 °C. ^1H NMR ($CDCl_3$) δ 8.47 (d, 1H, ArH); 8.16 (d, 1H, ArH); 7.79 (d, 1H, ArH); 7.61 (m, 1H, ArH); 7.52 (m, 2H, ArH); 6.72–6.82 (m, 3H, benzyl CH); 4.59 (s, 2H, benzyl CH_2); 3.78 (d, 6H, $2 \times OCH_3$) IR (KBr, cm^{-1}) 1517, 1234, 1139, 1023. Anal. $C_{18}H_{17}NO_2$ (279.339) calcd: N, 5.01; C, 77.40; H, 6.13. Found: N, 5.22; C, 77.45; H, 6.52.

6.12. 1-(3,4-Dimethoxybenzyl)-2-methyl-isoquinolinium iodide (11)

This product was obtained from compound **10** using the method described for compound **2** but the crude residue was used in the next step without further purification.

6.13. 1-(3,4-Dimethoxybenzyl)-2-methyl-1,2,3,4-tetrahydroisoquinoline hydrochloride (12)

$NaBH_4$ (5 equiv) was added to a solution of crude 1-(3,4-dimethoxybenzyl)-2-methyl-isoquinolinium iodide in ice-cold MeOH (20 mL). After 30 min the solution was evaporated under reduced pressure and the residue was dissolved in 1 N aqueous HCl (100 mL). The acidic solution was washed with Et_2O (2×30 mL) and then basified by addition of a 10% aqueous NaOH. The resulting suspension was extracted with CH_2Cl_2 (3×50 mL) and the organic layer was dried with anhydrous $MgSO_4$ before the evaporation of solvent under reduced pressure. The crude oil was isolated as an hydrochloride salt and was recrystallized in EtOH/Et_2O (24%). mp: 226–227 °C. ^1H NMR ($CDCl_3$) δ 7.32–7.27 (t, 1H, Ar*H*); 7.22–7.20 (d, 1H, Ar*H*); 7.13–7.08 (t, 1H, Ar*H*); 6.75–6.73 (d, 1H, Ar*H*); 6.64–6.60 (d, 2H, Ar*H*); 6.51–6.48 (d, 1H, Ar*H*); 4.29–4.13 (d, 1H, benzyl–C*H*–N); 3.97–3.92 (d, 1H, C_6H_3–CH_2–); 3.85 (s, 3H, OCH_3); 3.74 (s, 3H, OCH_3); 3.64 (m, 1H, C_6H_3–CH_2–); 3.34–3.30 (m, 1H, phenyl–CH_2–CH_2–N); 3.11–3.04 (m, 3H, phenyl–CH_2–CH_2–N); 2.87 (s, 3H, NCH_3). IR (*KBr*, cm^{-1}) 2934, 2467, 1510. Anal. $C_{19}H_{24}NO_2Cl$ (333.856) calcd: N, 4.20; C, 68.36; H, 7.25. Found: N, 4.42; C, 68.22; H, 7.96.

6.14. 1-(3,4-Dimethoxybenzyl)-2,2-dimethyl-1,2,3,4-tetrahydroisoquinolinium iodide (13)

Compound **13** was prepared using the experimental procedure described for compound **2** and was recrystallized from EtOH/Et_2O (55%). mp: 182 °C. ^1H NMR ($CDCl_3$) δ 7.26–7.19 (m, 2H, Ar*H*); 7.02–6.97 (m, 1H, Ar*H*); 6.71 (d, 1H, Ar*H*); 6.55 (d, 2H, Ar*H*); 6.47 (d, 1H, Ar*H*); 5.3 (m, 1H, benzyl–C*H*–N); 4.08 (m, 1H, phenyl–CH_2–CH_2–N); 3.88 (s, 3H, OCH_3); 3.85 (s, 3H, OCH_3); 3.74 (s, 4H, NCH_3 et phenyl–CH_2–CH_2–N); 3.66 (d, 1H, phenyl–CH_2–CH_2–N); 3.49 (s, 3H, NCH_3); 3.33–3.25 (m, 2H, C_6H_3–CH_2–); 2.97 (dd, 1H, phenyl–CH_2–CH_2–N). IR (KBr, cm^{-1}) 2933, 1517, 1025. Anal. $C_{20}H_{26}NO_2I$ (439.333) calcd: N, 3.19; C, 54.68; H, 5.97. Found: N, 3.35; C, 54.70; H, 6.33.

6.15. *N*-Methyl-laudanosine iodide (15)

A mixture of (±)-laudanosine **14** (0.1 g; 0.28 mmol) and an excess of methyl iodide (0,17 mL; 28 mmol) in acetonitrile (10 mL) was refluxed overnight. Excess of reagent and solvent were removed under reduced pressure. The crude quaternary salt was isolated in an acetone/diethylether mixture. Finally, the product was recrystallized from EtOH (0.115 g; yield: 82%). mp: 209–210 °C. ^1H NMR ($CDCl_3$) δ 6.89 (s, 1H, Ar*H*); 6.69–6.62 (d, 3H, Ar*H*); 6.46 (s, 1H, Ar*H*); 5.2 (d, 1H, –C*H*–N); 3.9–3.8 (m, 14H, $4 \times OCH_3$ and benzyl CH_2); 3.52 (s, 2H, phenyl–CH_2–CH_2–N); 3.38 (s, 8H, $2 \times NCH_3$ and phenyl–CH_2–CH_2–N). IR (KBr, cm^{-1}) 2920, 1515. Anal. $C_{22}H_{30}NO_4I$ (499.385) calcd: N, 2.80; C, 52.91; H, 6.06. Found: N, 3.03; C, 52.95; H, 6.14.

6.16. *N*-Ethyl-laudanosine iodide (16)

Compound **16** was prepared using the experimental procedure described for compound **15** with an excess of ethyl iodide in place of methyl iodide and was recrystallized from EtCOMe (0.112 g; yield: 78%). mp: 157–158 °C. ^1H NMR ($CDCl_3$) δ 6.87 (s, 1H, Ar*H*); 6.66–6.60 (m, 2H, Ar*H*); 6.45 (d, 1H, Ar*H*); 5.70 (s, 1H, Ar*H*); 5.01 (dd, 1H, –C*H*–N); 3.86–3.63 (m, 18H, $4 \times OCH_3$, phenyl–CH_2–CH_2–N and benzyl CH_2); 3.36 (s, 3H, NCH_3); 3.10 (q, 2H, CH_2CH_3); 1.41 (t, 3H, CH_2CH_3). IR (KBr, cm^{-1}) 2931, 1517. Anal. $C_{23}H_{32}NO_4I$ (513.412) calcd: N, 2.73; C, 53.81; H, 6.28. Found: N, 3.09; C, 53.95; H, 6.14.

6.17. *N*-Butyl-laudanosine iodide (17)

Compound **17** was prepared using the experimental procedure described for compound **15** with an excess of freshly distilled butyl iodide in place of methyl iodide and was recrystallized from Me_2CO/Et_2O (0.08 g; 53%). mp: 100–102 °C. ^1H NMR ($CDCl_3$) δ 6.89 (s, 1H, Ar*H*); 6.70–6.57 (m, 2H, Ar*H*); 6.45–6.42 (m, 1H, Ar*H*); 5.69 (s, 1H, Ar*H*); 5.03 (dd, 1H, –C*H*–N); 3.82–3.77 (m, 18H, $4 \times OCH_3$, phenyl–CH_2–CH_2–N and benzyl CH_2); 3.39 (s, 3H, NCH_3); 3.10 (t, 2H, $CH_2CH_2CH_2CH_3$); 1.78 (quintuplet, 2H, $CH_2CH_2CH_2CH_3$); 1.29 (sextuplet, 2H, $CH_2CH_2CH_2CH_3$); 0.89 (t, 3H, $CH_2CH_2CH_2CH_3$). *m/z* 414 (M^+). IR (KBr, cm^{-1})

2959, 1517, 1262. Anal. $C_{25}H_{36}NO_4I \cdot 3/4H_2O$ (554.979) calcd: N, 2.52; C, 54.05; H, 6.76. Found: N, 2.78; C, 54.28; H, 7.16.

6.18. *N*-Benzyl-laudanosine chloride (18)

Compound **18** was prepared using the experimental procedure described for compound **15** with an excess of benzyl chloride in place of methyl iodide and was recrystallized from CH_2Cl_2/Et_2O (0.22 g; yield: 80%). mp: 178–180 °C. ^1H NMR ($CDCl_3$) δ 7.50–7.36 (m, 5H, N–CH_2–C_6H_5); 6.68–6.64 (m, 2H, Ar*H*); 6.54 (s, 1H, Ar*H*); 6.37 (d, 1H, Ar*H*); 5.70 (s, 1H, Ar*H*); 5.08 (dd, 1H, benzyl–C*H*–N); 4.93 (d, 1H, N–CH_2–C_6H_5); 4.79 (d,1H, N–CH_2–C_6H_5) 3.85–3.79 (m, 12H, $4\times OCH_3$); 3.42 (s, 3H, NCH_3) 3.32–2.86 (m, 6H, phenyl–CH_2–CH_2–N and benzyl CH_2). *m*/*z* 448 (M^+). IR (KBr, cm^{-1}) 2938, 1518, 1264. Anal. $C_{28}H_{34}NO_4Cl \cdot 1/2H_2O$ (493.042) calcd: N, 2.82; C, 68.15; H, 7.30. Found: N, 3.03; C, 68.21; H, 7.16.

6.19. Racemic resolution of laudanosine

The enantiomers were separated from (±)-laudanosine **14** (600 mg) by chiral preparative HPLC using the following conditions: column CHIRACEL® OD 250×50 mm, 20 μm; eluent heptane/isopropanol/diethylamine 80/20/0.05; flow rate 120 mL/min; UV detection at 300 nm and room temperature. (+)-Laudanosine (300 mg) was firstly eluted at 7.7 min and (−)-laudanosine (300 mg) was eluted at 16.5 min.

6.20. (+)-*N*-Methyl-laudanosine iodide (19)

A mixture of (+)-laudanosine (0.25 g; 0.70 mmol) with an excess of methyl iodide (0,17 mL; 28 mmol) in acetonitrile (10 mL) was refluxed overnight. Excess of reagent and solvent was removed under reduced pressure. The crude quaternary salt was recrystallized from MeCN/Et_2O (0.31 g; yield: 89%). mp: 216–218 °C. $[\alpha]_D^{20}$: +95.0 Anal. $C_{22}H_{30}NO_4I$ (499.385) calcd: N, 2.80; C, 52.91; H, 6.06. Found: N, 2.97; C, 52.55; H, 6.15.

6.21. (−)-*N*-Methyl-laudanosine iodide (20)

The (−)stereoisomer was prepared using the experimental procedure described for compound **18**. The crude quaternary salt was recrystallized from MeCN/Et_2O yield: 80%. mp: 216–218 °C. $[\alpha]_D^{20}$: −86.7 Anal. $C_{22}H_{30}NO_4I$ (499.385) calcd: N, 2.80; C, 52.91; H, 6.06. Found: N, 3.12; C, 52.58; H, 6.10.

7. Biological evaluation

7.1. Radioligand binding studies and data analysis

7.1.1. Synaptosome preparation. Rats (male Wistar, ±250 g) were killed by decapitation and the brains were quickly removed and kept on ice during dissection. Crude cortex was dispersed in 0.32 M sucrose by using a Potter® homogeniser. After a first centrifugation at $1500\times g$ for 10 min, the supernatant was centrifuged at $25{,}000\times g$ for 10 min. The resulting pellet was dispersed in 5 mL 0.32 M sucrose to be aliquoted. Protein concentration was determined by the method of Hartree with bovine serum albumin as a standard.[32]

7.1.2. Binding experiments. The buffer consisted of a 10 mM Tris–HCl (pH 7.5) solution containing 5.4 mM KCl and 0.1% bovine serum albumin. The radioligand was ^{125}I-apamin (Perkin–Elmer, Specific activity 81.4 TBq $mmol^{-1}$). Glass fibre filters (Whatman GF/C) used in these experiments were coated for 1 h in 0.5% polyethylenimine and then washed with 2.5 mL of the ice-cold buffer just before use. Binding experiments were always terminated as follows. Aliquots were filtered under reduced pressure through Whatman filters. Filters were rapidly washed twice with 2.5 mL of buffer. The radioactivity remaining on the filter was evaluated with a Packard Tri-Carb 1600TR liquid scintillation analyser with an efficacy of 69%. ^{125}I-apamin binding to the filters was also estimated in the absence of synaptosomes. This binding was also subtracted from the total binding. Curve fitting was carried out using GraphPad Prism®.

Saturation binding experiments: Synaptosomes (0.2 mg of protein/mL) were incubated with increasing concentrations of ^{125}I-apamin (25 μL) with 975 μL of incubation buffer for 1 h at 0 °C. Samples were then filtered on Whatman GF/C filter and the radioactivity was measured as described above. Nonspecific binding was determined in parallel experiments in the presence of an excess of unlabeled apamin (0.1 μM) and subtracted from the total binding to obtain the specific binding.

Competition experiments between ^{125}I-apamin and drugs: Synaptosomes (0.2 mg of protein/mL) were incubated for 1 h at 0 °C with ±10 pM of ^{125}I-apamin (25 μL) and nine concentrations of drugs (10^{-4} to 10^{-7} M). Nonspecific binding was determined in the presence of an excess of unlabeled apamin (0.1 μM). Samples were then filtered on Whatman filter and the radioactivity was measured as described above.

7.2. Electrophysiological experiments

The procedure is largely described in a previous paper.[24] Briefly, male Wistar rats (150–200 g) were anaesthetised with chloral hydrate (400 mg/kg IP) and decapitated. The brain was excised quickly and placed in cold (~4 °C) artificial cerebro-spinal fluid (ACSF) at the following composition (in mM): NaCl 126, KCl 2.5, NaH_2PO_4 1.2, $MgCl_2$ 1.2, $CaCl_2$ 2.4, glucose 11, $NaHCO_3$ 18, saturated with 95% O_2 and 5% CO_2 (pH 7.4). A block of tissue containing the midbrain was cut in horizontal slices (thickness 350 μm) in a Vibratome (Lancer). The slice containing the region of interest was placed on a nylon mesh in a recording chamber (volume 500 μl). The tissue was completely immersed in a continuously flowing (~2 mL/min) ACSF, heated at 35 °C. Most recordings were made from dopaminergic neurones located in the substantia nigra pars compacta. Identification of dopaminergic cells was performed as described previously.[33] Intracellular recordings were performed

using glass microelectrodes filled with KCl 2 M (resistance 70–150 MΩ). All recordings were made in the bridge balance mode, using a npi SEC1L amplifier (Tamm, Germany). The accuracy of the bridge was checked throughout the experiment. Membrane potentials and injected currents were recorded on a Gould TA240 chart recorder and on a Fluke Combiscope oscilloscope. The Flukeview software was used for off-line analysis in most cases. Drug effects on the prominent apamin-sensitive AHP in dopaminergic neurones were quantified as the percent reduction of the surface area of the AHP (in mV s), which was blocked by a maximally active concentration of apamin (300 nM).[33] Averages of four sweeps were considered in all cases. The spontaneous firing of the neurones was usually reduced by constant current injection (−20 to −100 pA) in order to increase the amplitude of the AHP. Because the amplitude of the AHP is very sensitive to the firing rate, care was taken to compare all AHPs of one cell at the same firing rate. All drugs were applied by superfusion; complete exchange of the bath solution occurred within 2–3 min. Curve fitting was carried out using GraphPad Prism® and the standard equation: $E = E_{max}/[1 + (IC_{50}/x)^h]$, where x is the concentration of the drug and h the Hill coefficient. Numerical values are expressed as means ± SEM. Apamin (Sigma) and all other drugs were dissolved in water.

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References and notes

1. Sah, P. *Trends Neurosci.* **1996**, *19*, 150.
2. Vergara, C.; Latorre, R.; Marrion, N. V.; Adelman, J. P. *Curr. Opin. Neurobiol.* **1998**, *8*, 321.
3. Kolher, M.; Hirschberg, B.; Bond, C. T.; Kinzie, J. M.; Marrion, N. V.; Maylie, J.; Adelman, J. P. *Science* **1996**, *273*, 1709.
4. Stocker, M.; Krause, M.; Pedarzani, P. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 4662.
5. Bretschneider, F.; Wrisch, A.; Lehmann-Horn, F.; Grissmer, S. *Biophys. J.* **1999**, *76*, 2351.
6. Sah, P.; Faber, L. *Prog. Neurobiol.* **2002**, *66*, 345.
7. Stocker, M.; Pedarzani, P. *Mol. Cell. Neurosci.* **2000**, *15*, 476.
8. Messier, C.; Mourre, C.; Bontempi, B.; Sif, J.; Lazdunski, M.; Destrade, C. *Brain Res.* **1991**, *551*, 322.
9. Deschaux, O.; Bizot, J. C.; Goyffon, M. *Neurosci. Lett.* **1997**, *222*, 159.
10. Ikonen, S.; Riekkinen, P., Jr. *Eur. J. Pharmacol.* **1999**, *382*, 151.
11. Van der Staay, F. J.; Fanelli, R. J.; Blokland, A.; Schmidt, B. H. *Neurosci. Biobehav. Rev.* **1999**, *23*, 1087.
12. Fournier, C.; Kourrich, S.; Soumireu-Mourat, B.; Mourre, C. *Behav. Brain Res.* **2001**, *121*, 81.
13. Pedarzani, P.; Mosbacher, J.; Rivard, A.; Cingolani, L. A.; Oliver, D.; Stocker, M.; Adelman, J. P.; Fakler, B. *J. Biol. Chem.* **2001**, *276*, 9762.
14. Steketee, J. D.; Kalavas, P. W. *J. Pharmacol. Exp. Ther.* **1990**, *254*, 711.
15. Shepard, P. D.; Bunney, B. S. *Exp. Brain Res.* **1991**, *86*, 141.
16. Seutin, V.; Johnson, S. V.; North, R. A. *Brain Res.* **1993**, *630*, 341.
17. Vincent, J. P.; Schweitz, H.; Lazdunski, M. *Biochemistry* **1975**, *14*, 2521.
18. Zerrouk, H.; Lavaba-Djerbari, F.; Fremont, V.; Meki, A.; Darbon, H.; Mansuelle, P.; Oughuideni, R.; Van Rietschoten, J.; Rochat, H.; Martin-Eauclaire, M. F. *Int. J. Pept. Protein Res.* **1996**, *48*, 514.
19. Chen, J. Q.; Galanakis, D.; Ganellin, C. R.; Dunn, P. M.; Jenkinson, D. H. *J. Med. Chem.* **2000**, *43*, 3478.
20. Johnson, S. W.; Seutin, V. *Neurosci. Lett.* **1997**, *231*, 13.
21. Mohri, K.; Suzuki, K.; Usui, M.; Isobe, K.; Tsuda, Y. *Chem. Pharm. Bull.* **1995**, *43*, 159.
22. Cheng, Y.-X.; Dukat, M.; Dowd, M.; Fiedler, W.; Martin, B.; Damaj, M. I.; Glennon, R. A. *Eur. J. Med. Chem.* **1999**, *34*, 177.
23. Uff, B. C.; Kershaw, J. R.; Neumeyer, J. L. *Org. Syn.* **1977**, *56*, 19.
24. Scuvée-Moreau, J.; Liégeois, J.-F.; Massotte, L.; Seutin, V. *J. Pharmacol. Exp. Ther.* **2002**, *302*, 1176.
25. Seutin, V.; Johnson, S. W. *Trends Pharmacol. Sci.* **1999**, *20*, 268.
26. Granier, C.; Pedroso Muller, E.; Van Rietschoten, J. *Eur. J. Biochem.* **1978**, *82*, 293.
27. Habermann, E. *Pharmacol. Ther.* **1984**, *25*, 255.
28. Nohmi, M.; Kuba, K. *Brain Res.* **1984**, *301*, 146.
29. Cook, N. S.; Haylett, D. G. *J. Physiol.* **1985**, *358*, 373.
30. Hugues, M.; Duval, D.; Kitabgi, P.; Lazdunski, M.; Vincent, J.-P. *J. Biol. Chem.* **1982**, *257*, 2762.
31. Seutin, V.; Massotte, L.; Liégeois, J.-F.; Scuvée-Moreau, J. *Soc. Neurosci. Abstr.* **2002**, *28*, 340.5.
32. Hartree, E. F. *Anal. Biochem.* **1972**, *48*, 422.
33. Seutin, V.; Scuvée-Moreau, J.; Dresse, A. *Neuropharmacology* **1997**, *36*, 1653.