Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 3459–3464

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

AROMATIC ANALOGS OF ARCAINE INHIBIT MK-801 BINDING TO THE NMDA RECEPTOR

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Received 31 July 1998; accepted 19 October 1998

Abstract - Aromatic analogs of arcaine were shown to have inhibitory effects on the binding of the channel blocking drug [^{3}H]MK-801 to the NMDA receptor complex. The most potent compound of the series was an N,N'-bis(propyl)guanidinium which inhibited [^{3}H]MK-801 binding with an IC_{50} of 0.58 μM and an IC_{50} of 12.17 μM upon addition of 100 μM spermidine. The increase in IC_{50} upon addition of spermidine suggests competitive antagonism between the inhibitor and spermidine at the arcaine-sensitive polyamine site of the NMDA receptor complex.

Introduction

The NMDA subtype of excitatory amino acid receptor is a ligand-gated ion channel that has been shown to play a role in various forms of synaptic plasticity. Prolonged activation of the receptor has been implicated in epilepsy,[1] Parkinson's disease,[2] and ischemic neuronal cell death.[3] The NMDA receptor is activated by numerous endogenous and exogenous substances that act at specific regulatory sites on the receptor complex. The NMDA receptor complex is blocked at resting membrane potentials by Mg^{2+} and also contains a site in which zinc acts allosterically to inhibit the agonist response independently of membrane potential.[4,5] Other modulators of the NMDA receptor include glycine,[6] redox agents,[7] neurosteroids,[8] and channel blocking drugs such as, phencyclidine (PCP), (+)-5-methyl-10-11-dihydro-5*H*-dibenzo[*a,d*]cyclohepten-5,10-imine (MK-801), N-(1-[2-thienyl]cyclohexyl)piperidine (TCP), and ketamine.[9-11] A last important family of modulators for the NMDA receptor are the polyamines, which bind to several distinct sites on the receptor.[12,13]

Polyamines have multiple effects on NMDA receptor activation and binding of channel blocking drugs. They increase the affinity of glycine at strychnine-insensitive glycine receptors,[14,15] and enhance the potency of glycine to increase NMDA-mediated currents.[16] Polyamines may act as agonists or antagonists (as defined by radioligand binding assays), depending on the polyamine concentration as well as its chemical structure.[17,18] The endogenous polyamines, spermine [*N,N'*-bis(3-aminopropyl)-1,4-butanediamine] and spermidine [*N*-(3-aminopropyl)-1,4-butanediamine], have been shown to stimulate the binding of [^{3}H]MK-801 to the NMDA receptor at both low and saturating levels of glutamate and glycine.[12]

However, spermine and spermidine inhibit [^{3}H]MK-801 binding at concentrations above 100 μM, producing a biphasic concentration response curve.[19] Other polyamines such as arcaine (1,4-diguanidino-butane) act to decrease [^{3}H]MK-801 binding at low concentrations therefore are classified as antagonists,[20] while related amidines and guanidines, such as pentamidine, inhibit [^{3}H]MK-801 binding independently of the

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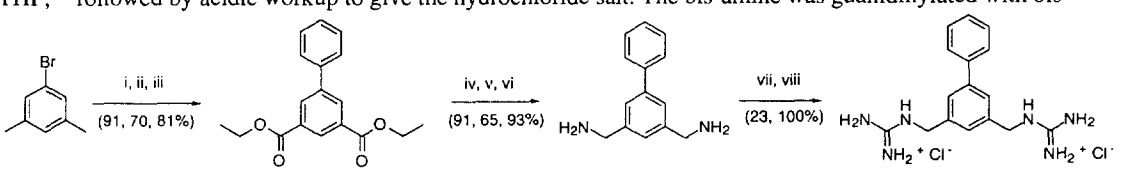
polyamine site.[21-23] The therapeutic potential of polyamine antagonists is not entirely clear. However, a polyamine antagonist such as arcaine that decreases [^{3}H]MK-801 binding could have therapeutic benefits by decreasing the binding affinity of PCP and so inhibiting its effects on the NMDA receptor. The potential of the polyamine site as a target for drug design suggests the need for a better understanding of polyamine activity at the NMDA receptor.

There has been work in the recent literature exploring the complex effects of synthetic polyamines on the NMDA receptor. Bergeron has carried out a structure activity relationship (SAR) study of the polyamine binding site with analogs of spermine, homospermine, and norspermine to determine the ideal chain length, number of charges, and nitrogen substitution necessary for polyamine activity.[24] The potential of heterocyclic compounds to act as polyamine site ligands was determined by the synthesis of *N,N'*-substituted piperizine and homopiperizine derivatives.[25] Other work has shown that the amino-1-oxy analog of spermidine has antagonist activity to spermine potentiation.[26] Pentamidine analogs that inhibit [^{3}H]MK-801 binding in the absence of spermidine have also been recently reported.[22] In this paper we describe the design and synthesis of several aromatic analogs of arcaine that act as inhibitors of [^{3}H]MK-801 binding. Previously, compound **1** was shown to have an inhibitory effect on [^{3}H]MK-801 binding in a manner similar to arcaine.[27] A series of related compounds was synthesized and assayed for [^{3}H]MK-801 inhibition at the NMDA receptor.

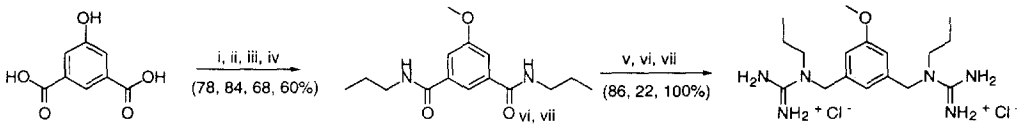
Methods:

A representitive synthesis of the monosubstituted bis-guanidinium derivatives is shown in Scheme 1. *N,N'*-bis[bis(amino)methylidenyl]-3,5-bis(aminomethyl)biphenyl dichloride (**5**) was prepared starting from 5-bromo-*m*-xylene via oxidation to 5-bromoisophthalic acid with $KMnO_4$, followed by esterification with ethanol and sulfuric acid. The resulting 5-bromoethyl isophthalate was used in Suzuki coupling[28] with phenylboronic acid and Pd(II)tetrakis-triphenylphosphine. The product biphenyl diester was reduced to the diol with $LiAlH_4$ in THF, which was converted to the bis-azide by a Mitsunobu-type reaction[29] with diphenylphosphorylazide (DPPA) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU). The bis-azide was reduced to the bis-amine with H_2/Pd-C in methanol[30] followed by guanidinylation with bis-(Boc)thiourea in the presence of mercuric chloride[31,32] to give the bis-(Boc)protected guanidine. Deprotection of the *(tert)*- butoxycarbonyl groups to give the guanidinium was achieved with HCl gas in ethyl acetate.[33]

A second route was developed for disubstituted guanidiniums as is exemplified in Scheme 2 with *N,N'*-bis[bis-(amino)methylidenyl]-*N''*-bis[propyl]-1,3-bis(aminomethyl)-5-methoxybenzene dichloride (**13**). 5-Hydroxyisophthalic acid was esterified and then alkylated with methyl iodide to give bis-methyl 5-methoxyisophthalate. This was hydrolized with LiOH to the diacid followed by acid chloride formation with oxalyl chloride and reaction with n-propylamine. Reduction of the resulting amide was achieved with borane-THF,[34] followed by acidic workup to give the hydrochloride salt. The bis-amine was guanidinylated with bis-



Scheme 1: (i) $KMnO_4$/pyridine/H_2O, reflux; (ii) EtOH, H_2SO_4, reflux; (iii) phenylboronic acid, Pd(II)tetrakis-triphenylphosphine; (iv) $LiAlH_4$, THF; (v) diphenylphosphorylazide (DPPA), 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), DMF; (vi) H_2 Pd/C, methanol; (vii) bis-(Boc)thiourea, Et_3N, DMF; (viii) HCl (gas), ethyl acetate.



Scheme 2: (i) methanol, H_2SO_4; (ii) CH_3I, acetone, K_2CO_3; (iii) LiOH, THF/H_20; (iv) a. oxalyl chloride, CH_2Cl_2; b. n-propylamine, THF; (v) a. BH_3- THF; b. HCl/methanol; (vi) bis-(Boc)thiourea, Et_3N, $HgCl_2$; (vii) HCl gas, ethyl acetate.

(Boc)thiourea.[31,32] and deprotected with HCl gas/ethyl acetate[33] to give the bis-guanidinium derivative.

[^{3}H]Dizocilpine ligand binding assays were performed in well washed rat brain membranes as previously described.[35] Assays were performed in a final volume of 1 mL of 10 mM HEPES-NaOH (pH 7.4) that contained 0.2 mg protein, 0.5 nM [^{3}H]dizocilpine, 100 μM glutamate, 30 μM glycine along with the appropriate bis-guanidiniums in concentrations ranging from 100 nM to 100 μM. Nonspecific binding was determined by 30 μM dizocilpine. Assays were incubated for 2 h at room temperature and terminated by filtration (3 rinses) over Schleicher and Schuell #32 glass-fiber filters using a 24-well cell harvester (Brandel Inc.). Radioactivity was measured using a liquid scintillation counter at an efficiency of about 63%.

Results and Discussion:

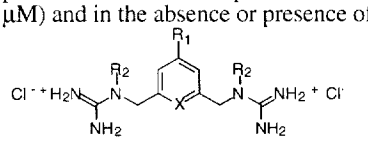
The arcaine analogs tested were all effective inhibitors of [^{3}H]MK-801 binding to the NMDA receptor complex (i.e., IC_{50}s under 25 μM). Arcaine [1,4-diguanidinobutane] has an IC_{50} value of 9.13 μM for the inhibition of [^{3}H]MK-801 binding in the presence of saturating concentrations of glutamate and glycine. The addition of 100 μM spermidine resulted in an increased IC_{50} of 79.98 μM. An increase in the IC_{50} of at least ten fold upon addition of spermidine suggests that the interaction is competitive between spemidine and arcaine for the same polyamine binding site.[20] Likewise, the effect of spermidine on the activity of the arcaine analogs was used to determine the nature of the polyamine interaction. In previous work we identified compound **1** (see Table 1) as a potential leadwith an IC_{50} of 1.19 μM and 7.09 μM upon addition of 100 μM spermidine.[27] In the present paper we have prepared derivatives of **1** to assess their recognition by the receptor and their subsequent effects on [^{3}H]MK-801 binding. The modifications included substitution on the benzene ring, alkylation of the guanidinium nitrogens, and replacement of the central spacer by a pyridine ring.

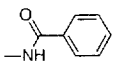
Five different derivatives of **1** were synthesized, each with a functional group on the benzene ring. A series of ether derivatives was prepared including compounds **2**, **3**, and **4**. Increasing the size of the alkoxy group from methoxy to cyclohexyloxy resulted in decreased potency. Methoxyguanidinium **2** has an IC_{50} of 1.30 μM. Isopropylguanidinium **3** has an IC_{50} of 2.43 μM, while cyclohexylguanidinium **4** has an IC_{50} of 9.07 μM. Phenylguanidinium **5** and benzamideguanidinium **6** were also less potent than **1** with IC_{50}s of 5.14 μM and 4.34 μM, respectively. The more hydrophobic isopropyl and cyclohexyl ethers resulted in decreased potency and lack of competition for the arcaine-sensitive polyamine site. This suggests that a hydrophobic group on the benzene ring is not favorable. The increases in IC_{50} upon the addition of 100 μM spermidine were not sufficient to suggest a competition between bis-guanidiniums **2-6** and spermidine for the same polyamine site.

Substitution on the guanidinum nitrogens resulted in a compound which inhibited [^{3}H]MK-801 binding more effectively than any previously known compounds. *N,N'*-bis(propyl)guanidinium **8** was the most potent of this series with an IC_{50} of 0.58 μM. Futhermore, the IC_{50} upon addition of 100 μM spermidine is 12.17 μM. This 21-fold increase suggests that *N,N'*-bis(propyl)guanidinium **8** is competitive with spermidine, as

shown in Figure 1. In the presence of 100 μM spermidine, *N,N'*-bis(propyl)guanidinium **8** concentration curve shifts to the right, suggesting *N,N'*-bis(propyl)guanidinium **8** is altering [^{3}H]MK-801 binding by acting at the arcaine-sensitive polyamine site (Figure 1). Variations in chain length led to higher IC_{50}s : *N.N'*-bis(ethyl)guanidinium **7** (IC_{50} 5.10 μM), *N,N'*-bis(butyl)guanidinium **10** (IC_{50} = 2.80 μM), *N,N'*-bis(isobutyl)guanidinium **11** (IC_{50} =10.83 μM). The substitution of aromatic and alkene functional groups on the guanidinium nitrogens resulted in less potent compounds than *N, N'*-bis(propyl)guanidinium **8**. *N,N'*-bis(allyl)guanidinium **9** and *N,N'*-bis(benzyl)guanidinium **12** have IC_{50}s of 3.85 μM, and 3.82 μM respectively. Substitution on the guanidinium nitrogens is favorable for [^{3}H]MK-801 inhibition. The optimal chain length from our experiments is the 3 carbon propyl chain. Another important point is the difference in potency between the propyl and allyl chains on the guanidinium nitrogens. The carbon number of these chains is the same, but the presence of the double bond instead of an alkyl chain led to a 4-fold loss of activity. Of the latter bis-substituted guanidiniums, only *N,N'*-bis(ethyl)guanidinium **7** showed competition with spermidine.

Table 1. Effects of arcaine analogues on [^{3}H]MK-801 binding. IC_{50} values from [^{3}H]MK-801binding assays represent the mean (±S.E.M.) of three experiments performed in duplicate. Assays were performed under non-equilibrium conditions in the presence of saturating L-glutamate (100 μM) and glycine (30 μM) and in the absence or presence of a saturating spermidine concentration as indicated.



	R1	R2	X	IC_{50} (μM)	IC_{50} (μM)+100 μM spermidine
arcaine				9.13 ± 1.12	79.98 ± 10.99
1	H	H	CH	1.19 ± 0.012	7.09 ± 0.70
2	-OCH_3	H	CH	1.30 ± 0.13	5.89 ± 0.85
3		H	CH	2.43 ± 0.18	10.47 ± 0.59
4		H	CH	9.07 ± 0.34	9.88 ± 0.60
5		H	CH	5.14 ± 0.16	15.71 ± 0.78
6		H	CH	4.34 ± 0.34	20.56 ± 0.76
7	H	-CH_2CH_3	CH	5.10 ± 0.22	75.8 ± 14.66
8	H	-$CH_2CH_2CH_3$	CH	0.58 ± 0.15	12.17 ± 0.29
9	H		CH	3.85 ± 0.30	29.81 ± 2.43
10	H	-$CH_2CH_2CH_2CH_3$	CH	2.80 ± 0.37	9.27 ± 1.82
11	H		CH	10.83 ± 0.83	112.03 ± 13.89
12	H		CH	3.82 ± 0.54	2.23 ± 0.48
13	-OCH_3	-$CH_2CH_2CH_3$	CH	20.6 ± 0.84	55.26 ± 14.60
14	-OH	-$CH_2CH_2CH_3$	CH	10.60 ± 0.98	110.5 ± 5.48
15	H	H	N	4.02 ± 0.79	49.30 ± 3.25
16	H	-$CH_2CH_2CH_3$	N	8.62 ± 0.70	95.21 ± 5.73

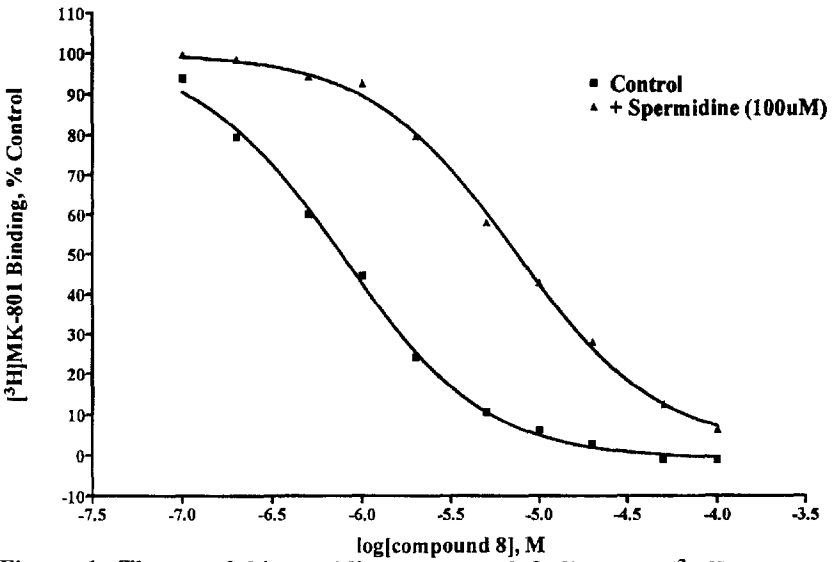


Figure 1. The novel bisguanidine compound 8 displaces [^{3}H]MK-801 specific binding while acting at the arcaine-sensitive polyamine site. Compound **8** concentration-dependently decreases [^{3}H]MK-801 specific binding in the absence (■) or presence (▲) of 100 μM spermidine. In the presence of 100 μM spermidine, the concentration curve for **8** shifts to the right, suggesting it is altering MK-801 binding by acting at the arcaine-sensitive polyamine site. The data shown is a representative experiment performed in duplicate and repeated three times.

Two compounds with substituents both on the benzene ring, and on the guanidinium nitrogens were made. *N,N'*-bis(propyl)methoxyguanidinium **13** was made to examine if the inhibition properties of a methoxy group on the benzene ring and bis(propyl) substitution on the guanidinium nitrogens were additive. *N,N'*-bis(propyl)methoxyguanidinium **13** was neither a potent nor a competitive inhibitor of [^{3}H]MK-801 binding with an IC_{50} of 20.6 μM and 55.26 μM upon addition of spermidine. *N,N'*-bis(propyl)hydroxylguanidinium **14** was slightly more potent with an IC_{50} of 10.60 μM. However, compound **14** was competitive with spermidine as shown by the IC_{50} of 110.53 μM upon addition of spermidine. A possible explanation for the lack of activity of these two compounds is that the benzene substituted bis-guanidiniums (**2-6**), and the guanidinium nitrogen substituted compounds (**7, 9-12**) bind to different sites on the receptor. The last two compounds synthesized contain pyridine rings. Pyridine guanidinium **15** has the same ability to inhibit [^{3}H]MK-801 as lead compound **1**, but with a 12-fold increase in IC_{50} upon the addition of spermidine. The IC_{50s} were 4.02 μM and 49.30 μM upon addition of spermidine. The corresponding N-alkylated compound, *N,N'*-bis(propyl)guanidinium **16**, was less potent with an IC_{50} of 8.62 μM and 95.21 μM upon addition of spermine. This was unexpected because the corresponding benzene ring compound, *N,N'*-bis(propyl)-guanidinium **8**, was more potent than the parent compound **1**.

In conclusion, this work gives information on the features of bis-guanidiniums that influence [^{3}H]MK-801 binding at the NMDA receptor. Important structural features include the location and nature of the functional groups. The most potent of the compounds synthesized was *N,N'*-bis(propyl)guanidinium **8**. This compound is interesting because it demonstrates that an alkyl substituted terminal basic group can cause a greater inhibition

of [^{3}H]MK-801 binding to the NMDA receptor than the parent compound arcaine. The spacer between the guanidiniums also plays a role in determining the activity toward [^{3}H]MK-801 inhibition, as shown by substitution of the benzene ring by a pyridine ring.

These studies also show that minor alterations in the structure of these compounds alter their selectivity for the arcaine-sensitive polyamine site. For example, the comparison of **13** and **14** reveals a substantial difference in the effect of adding spermidine to the assay conditions. The nature of the spermidine-insensitive site that contributes to the effects of compounds **2-6**, **10**, **12**, and **13** is not known, although we have reported that pentamidine and 1,10-diguanidinodecane act at a polyamine-insensitive site. These findings illustrate the need for caution in interpreting SAR data in the absence of information of pharmacological specificity.

Acknowledgments. We thank the National Institutes of Health (DA07409 to IJR. and GM53579 to ADH) for financial support of this work.

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