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Natural and synthetic polyamines: modulators of signalling proteins

Peter N.R. Usherwood

Division of Molecular Toxicology, School of Biological Sciences, University of Nottingham, Nottingham, NG7 2RD, UK

Abstract

Ionotropic receptors are modulated allosterically by natural polyamines, such as spermine, and by polyamine derivatives, such as polyamine amides (e.g. philanthotoxin-343) and polymethylene tetraamines (e.g. methoctramine. Modulation can be either positive (potentiation) or negative (non-competitive antagonism of either open or closed channel receptor conformation). Photoaffinity labelling studies have identified a site close to the channel lumen on the nicotinic acetylcholine receptor *Torpedo* electroplax that is probably the allosteric site responsible for antagonism of the closed channel conformation of this receptor.

Keywords: Polyamines; Signalling proteins; Neurotoxins

Like amino acids, proteins and nucleic acids, polyamines are present in all animal cells at high concentrations. Amongst their multifunctional biological properties is their modulation of protein function, including that of neuroactive proteins such as transmitter receptors. The first studies of brain polyamines were reported in the 1970s [1], but polyamines did not become a major focus of neuroscience research until many years later. It was the discovery of polyamine-containing neurotoxins in the venom of certain spiders and wasps in the 1980s that alerted neuroscientists to the possible role of exogenous polyamines as modulators of ionotropic transmitter receptors [2]. When Ransom and Stec [3] described the effects of spermine (**1**) and spermidine in the binding of ligands to *N*-methyl-D-aspartate (NMDA) receptors, a class of ionotropic glutamate receptor, and Brackley et al. [4] showed electrophysiologically that spermine (**1**) potentiated (at low concentrations) and inhibited (at high concentrations) the currents elicited by agonist activation of ionotropic glutamate receptors, it became clear that polyamines also modulate neurotransmitter receptor function. Previously, studies of metabotropic transmitter receptors had shown that benextramine, a polymethylene tetraamine, not only covalently inhibits α-adrenergic receptors, but also competitively antagonises muscarinic acetylcholine receptors [5]. These discoveries were not exploited until much later when it was found that polymethylene tetraamines also target ionotropic acetylcholine receptors [6]. The present decade has seen an increasing interest in natural and synthetic polyamines and polymethylene–tetraamines as exogenous modulators of ionotropic transmitter receptors, and a growing realisation of the important roles played by endogenous polyamines in the nervous system.

Natural polyamines, such as spermine (**1**), spermidine and putrescine are simple aliphatic amines containing 2–3 flexible carbon chains separated by secondary amines with primary amines at either end. The amino groups are strongly basic and fully protonated at physiological pH (7.4). Electrostatic interactions of polyamines with anionic sites on macromolecules such as transmitter receptor proteins, is the mechanistic basis for the neurological functioning of these compounds. Spermine and spermidine potentiate and inhibit NMDA receptors by interacting with extracellular sites and ion channel sites, respectively. Although the extracellular concentrations of these polyamines are probably high enough in vivo for interaction with their potentiating sites on NMDA receptors, they are probably too low to inhibit these proteins. However, following brain injury or a stroke the extracellular concentrations of polyamine may rise to inhibitory levels. Some transmitter receptors are modulated intracellularly by polyamines. Although most intracellular polyamine is bound, the free concentrations of these

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compounds are normally sufficient to modulate these receptors. Because spermine (**1**), spermidine and putrescine have low affinities for ionotropic receptors ($\gg \mu M$), their sites of interaction with these proteins are difficult to define in binding studies. Binding to these sites is also masked by non-specific interactions. However, polyamine-containing spider and wasp toxins (polyamine amides) and polymethylene tetraamines have much higher affinities for these sites and, as such, are excellent tools for indirectly characterising polyamine interactions with transmitter receptors [2].

Polyamine amides usually have an aromatic moiety (head group) that may also carry a side chain (butyryl in the case of PhTX-343 (**2**) (the digits refer to the number of methylene groups separating the amines of the polyamine moiety starting from the aromatic end of the molecule)). These compounds may contain one or more amino acids (e.g. tyrosine, asparagine) [7].

Hundreds of natural and synthetic polyamine amides are available for SAR studies (e.g. [8]). Many syntheses of these compounds have been based on a toxin, philanthotoxin-433, isolated from a wasp, *Philanthus triangulum* [9]. Most SAR studies have been based on PhTX-343 (**2**), which contains spermine (**1**). In view of their polycationic properties, it is unsurprising that polyamine amides are best known for their antagonism of the open channel states of ionotropic receptors that gate cation-selective ion channels. However, this is misleading because their affinity for the open channel conformations of such receptors is often low. Despite this, it is clear that they are very potent antagonists of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptors, a class of non-NMDA-type ionotropic glutamate receptors, and nicotinic acetylcholine receptors (nAChR), which are excitatory receptors and gate intrinsic cation-selective ion channels. At low concentrations ($\sim$ nM), polyamine amides potentiate these receptors, although receptor subunit composition may qualitatively or quantitatively influence this phenomenon. Micromolar concentrations of polyamine amides cause non-competitive antagonism through allosteric inhibition of the closed channel states of these receptors. It is only at $>\mu M$ concentrations that polyamine amides are open channel blockers. Finally, intracellular application of PhTX-343 (**2**) (and spermine (**1**)) at μM concentrations may non-competitively inhibit kainate-type ionotropic glutamate receptors (GluR6 [10]) and neuronal nAChR [11,12], but not muscle nAChR expressed in BC_3H1 cells [13]). Is it possible to dissect out the different actions of polyamine amides? A step in the direction of separating the inhibitions of closed and open channel conformations has been made by studying the action of PhTX-343 (**2**) on recombinant AMPA receptors containing GluR2 subunits — the presence of an arginine in the channel lining domain of the GluR2 subunit prevents access of a polyamine amide to the channel and, thereby, prevents open channel block [14]. Also, the anion selective ion channel gated by a recombinant A-type γ-aminobutyric acid receptor is not blocked by PhTX-343 (**2**), but activity of this receptor is modulated allosterically (inhibited) by interaction of the polyamine amide with its closed channel conformation [4].

Until recently, most of the SAR studies were undertaken without an understanding of the multiple sites of action of these compounds and, so far, there have been no SAR studies on the potentiating sites on ionotropic receptors. Despite these deficiencies, it is clear that for antagonism of both open and closed channel receptor conformations, the terminal aromatic moiety has a major influence on potency, and the size and hydrophobicity of this moiety is an important parameter. This is also true for the polyamine moiety; its length and its number of positively-charged groups having a major influence on inhibition of both closed and open channel conformations of a receptor. Although spermine is only a weak antagonist of ionotropic receptors, the incorporation into this molecule of certain aromatic moieties without side-chains increases potency above that for PhTX-343 (**1**) [19]. However, the side-chain is not redundant, because polyamine amides with very hydrophobic side-chains are amongst some of the most potent antagonists in this class [8]. SAR studies have shown that the size of a head group influences potency, although regional differences in head group hydrophobicity might also be contributory factors [8]. The minimum diameter of the cation-selective channel gated by an ionotropic receptor should, in principle, introduce potency differences between polyamine amides that can and cannot permeate the channel and, in the latter case, between those with long and short polyamine moieties.

Potentiation. Spermine potentiates responses of nAChR of the TE-671 muscle cell line [15] and NMDA receptors [16] by reducing the impact of desensitisation. In both cases, arcaine competitively inhibits the allosteric action of spermine. PhTX-343 also potentiates these receptors and AMPA receptors, although it is not known whether this involves an inhibition of desensitisation. It is interesting that despite the fact that the affinity of a polyamine amide for this site is much higher than that for the two extracellular inhibitory sites [17] a separate binding site for potentiation of nAChR by polyamine amide was not identified in the photolabelling studies of Bixel [18] (see below).

Antagonism of the closed channel receptor conformation. Using a laser pulse photolysis technique, Jayaraman et al. [13] investigated the kinetics of binding of PhTX-343 to nAChR of BC_3H1 cells. The affinity of the polyamine amide for the closed channel conformation of this receptor was found to be five times greater than that for the open channel. Further to elucidate the binding of polyamine amides (and polymethylene te-

traamines) to nAChR, a photolabile, radioactive (^{125}I), azido hybrid of PhTX-343 (**2**) and methoctramine (**3**) has been photo-cross-linked to nAChR-rich membranes obtained from *Torpedo* electroplax. Previously, it had been shown that fluorescent ethidium, which is a non-competitive antagonist of nAChR, displaces the unexcited photolabel from nAChR [18] and in electrophysiological studies the unexcited photolabel exclusively antagonised the closed channel conformation of this receptor. When excited, the photolabile polyamine amide predominately labelled the α-subunits of nAChR, most of the label being localised to a 1.7 kDa peptide beginning at αSer162, which is part of the extracellular domain of the α-subunit. The short sequence of amino acids carries five negative charges and is thought to be located near to the channel lumen, but also to overlap with the agonist binding site. Previously, the polyamine amide N_3-phenyl-$^{125}I_2$-PhTX-343-lysine was photo-cross-linked to *Torpedo* nAChR [19]. Again, the label was mainly confined to the α-subunit, being predominantly found in a 20 kDa fragment comprising Serl73 to Glu338 and to a lesser extent in a 12 kDa fragment Asn339-Gly437. SAR studies have shown that the presence of the aromatic head group in a polyamine amide raises its inhibitory potency well above that of spermine, suggesting that this part of the molecule may fit into a hydrophobic pocket of nAChR during antagonism of its closed channel conformation. However, it is clear that the polyamine moiety is also important for antagonism of the closed channel receptor state of nAChR because tyrosyl-butyryl is a much weaker antagonist of this state than PhTX-343 (**2**).

Open channel block. Polyamines are small polycations that permeate cation-selective ion channels gated by ionotropic receptors, provided that the subunit composition of these proteins does not prevent their access to the channels. It has been assumed that the polyamine moiety of a polyamine amide adopts an extended conformation under physiological conditions due to repulsion of its protonated amino groups. Jaroszewski et al. [20] have recently obtained NMR evidence to support this assumption. Although it has not been shown directly, it is also assumed that the uncharged moiety of PhTX-343 (**1**) is probably flexible, but that in an aqueous environment it does not form internal hydrogen bonds with the polyamine moiety. Polyamine amides with small aromatic head groups are also permeant [21] yet they are more potent open channel blockers than polyamines. What accounts for this difference? Open channel block may arise when the head group of a polyamine amide binds to a hydrophobic pocket near the external orifice of the channel and the polyamine moiety extends into the channel where it binds to nucleophilic carbonyl or hydroxyl groups. Perhaps the hydrophobic binding site is the same for antagonism of closed and open channel states. Presumably, spermine (**1**) also channel blocks by binding to nucleophilic groups in the channel, but its affinity is much lower (>100 times) than that of a polyamine amide because it does not have an aromatic head group as a 'holdfast'. How many nucleophilic groups are there in a receptor channel, how distant are they from the extracellular polyamine binding site and how are they distributed? It is likely that there is an array of nucleophilic groups and that the number of such groups engaged by a polyamine amide is determined by the number of positively-charged sites on the polyamine moiety of the polyamine amide and the spatial distribution of these sites. By varying the inter-nitrogen distances in the polyamine moiety it should be possible to estimate the distance between the nucleophilic groups, because compounds with an inter-nitrogen distance equal to or close to the distance between nucleophilic groups must be much more potent than others.

An objective of studies of polyamine-containing compounds on the nervous system is to discover analogues of PhTX-343 (**2**) and methoctramine (**3**) that are valuable commercially, for example as neuroprotectants [22]. Also, the inhibitory regulatory site on the closed channel conformation of nAChR [13] to which PhTX-343 (**2**) binds is also a binding site for cocaine. Therefore, a polyamine amide that displaces cocaine in vivo might alleviate the toxic affects of the latter.

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References

[1] G.G. Shaw, Arch. Int. Pharmacodyn. Ther. 198 (1972) 36–48.
[2] H. Jackson, P.N.R. Usherwood, Trends Neurosci. 11 (1988) 263–278.
[3] R.W Ransom, S.L. Stec, J. Neurochem. 51 (1988) 830–836.
[4] P Brackley, R. Goodnow Jr., K. Nakanishi, H.L. Sudan, P.N.R. Usherwood, Neurosci. Lett. 114 (1990) 51–56.
[5] C. Melchiorre, M.S. Yong, B.C. Benley, B. Belleau, J. Med. Chem. 21 (1978) 1126.
[6] C. Melchiorre, Trends Pharmacol. Sci. 9 (1988) 216–220.
[7] P.N.R. Usherwood, I.S. Blagbrough, Pharmacol. Ther. 52 (1991) 245–268.
[8] K. Nakanishi, S.-K. Choi, D. Hwang, K. Lerro, M. Orlando, A.G. Kalivretenos, A. Eldefrawi, M. Eldefrawi, P.N.R. Usherwood, Pure Appl. Chem. 86 (1994) 671–678.

[9] A.T. Eldefrawi, M.E. Eldefrawi, K. Konno, N.A. Mansour, E. Nakanishi, E. Oltz, P.N.R. Usherwood, Proc. Natl. Acad. Sci. USA 65 (1988) 4910–4913.
[10] R. Bahring, D. Bowie, M. Benveniste, M. Mayer, J. Physiol. 502.3 (1997) 575–589.
[11] A.P. Haghigi, E. Cooper, J. Neurosci. 18 (1998) 4050–4062.
[12] M. Liu, K. Nakazawa, K. Inoue, Y. Ohno, Br. J. Pharmacol. 122 (1997) 379–385.
[13] V. Jayaraman, P.N.R. Usherwood, G.P. Hess, Biochemistry 38 (1999) 11406–11414.
[14] P.T.H. Brackley, D.R. Bell, S.-K. Choi, K. Nakanishi, P.N.R. Usherwood, J. Pharm. Exp. Ther. 266 (1993) 1573–1580.
[15] Z. Shao, I.R. Mellor, M.J. Brierley, J. Harris, P.N.R. Usherwood, J. Pharm. Exp. Ther. 286 (1998) 1269–1276.
[16] J. Lerma, Neuron 8 (1992) 343–352.
[17] I.S. Blagbrough, M. Bruce, B.W Bycroft, A.J. Mather, P.N.R. Usherwood, Pesticide. Sci. 30 (1990) 397–403.
[18] M.G. Bixel, M. Krauss, Y. Liu, M.L. Bologensi, M. Rosini, I.R. Mellor, P.N.R. Usherwood, C. Melchiorre, K. Nakanishi, F. Hucho, Eur. J. Biochem. 267 (2000) 110–120.
[19] S.-K. Choi, A.G. Kalivetrenos, P.N.R. Usherwood, K. Nakanishi, Chem. Biol. 2 (1995) 23–32.
[20] J.W. Jaroszewski, L. Matzen, B. Frölund, P. Krogsgaard-Larsen, J. Med. Chem. 39 (1996) 515–521.
[21] P.N.R. Usherwood, I.S. Blagbrough, in: C. Carter (Ed.), The Neuropharmacology of Polyamines, Academic Press, London, 1994, pp. 185–204.
[22] A.C. Green, K. Nakanishi, P.N.R. Usherwood, Brain Res. 717 (1996) 135–146.