Hypothesis

RECEPTOR OCCUPANCY DOSE–RESPONSE CURVE SUGGESTS THAT PHOSPHATIDYLINOSITOL BREAKDOWN MAY BE INTRINSIC TO THE MECHANISM OF THE MUSCARINIC CHOLINERGIC RECEPTOR

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Received 26 May 1976

Formation of cAMP has been characterised as an enzyme-catalysed reaction involved in the amplification of the chemical signals which are detected by various types of cell surface receptors [1], but little progress has been made towards identification of analogous reactions linked to receptors which do not exert their effects through control of adenylate cyclase. In some cases such reactions may not exist (e.g. the nicotinic cholinergic receptor? [2]) but in others it seems more likely that they have simply eluded identification by the experiments done so far. In most investigations of receptor mechanisms emphasis has been placed on responses which are provoked maximally by low ('physiological') concentrations of agonists. We would like to suggest that it might be more appropriate to concentrate on those responses which occur at higher, so-called 'unphysiological', agonist concentrations. This is because with many agonists (particularly small molecules such as neurotransmitters) it is necessary for only a small proportion of the cell's large population of functionally equivalent receptors to interact with agonist in order to provoke a maximal 'physiological' response such as secretion or contraction. This is thought to be because activation of only this small fraction of the total receptor population is sufficient to raise the intracellular concentration of a second messenger such as cyclic AMP or Ca^{2+} enough to stimulate the responsive enzyme(s) fully. High concentrations of agonists are, though, needed to bring about a maximum increase in the second messenger concentration. Thus, if the reaction which causes the change in concentration of a second messenger (for example, adenylate cyclase or the mechanism which opens cell-surface Ca^{2+} gates) is directly controlled by the activated fraction of the receptor population then its dose–response curve will follow the receptor occupation curve, i.e. that describing the agonist–receptor interaction. In a cell responding to an agonist for which there is a large population of spare receptors any reaction intrinsic to the mechanism which initiates amplification of the message received by the receptor should therefore show a dose–response curve displaced to much higher agonist concentrations than are needed for the 'physiological' responses. This general idea arises naturally from receptor occupation theory; it has already been used effectively in explaining the observed behaviour of receptor systems which involve control of adenylate cyclase and in which maximum activation of adenylate cyclase requires far higher agonist concentrations than are needed for maximum activation of 'physiological' responses [3–5]. Furthermore, recent studies have shown that the concentration of receptors of a particular type can vary widely from tissue to tissue (and at different times in a single tissue), but that the dose–response relationship which describes the agonist–receptor interaction is essentially identical in all systems in which it is measured (e.g., refs. [6–8]). As a result of the differences in receptor concentration, cell responses which are not directly coupled to the receptor vary in agonist sensitivity from tissue to tissue. In contrast, it is to be expected that reactions closely coupled to the activated receptors would vary in maximum response from tissue to tissue, but should always show dose–response curves identical with the receptor occupation curve: this is the pattern seen

with β-adrenergically controlled adenylate cyclase [7,9,10].

A potentially valuable application of these ideas, and one which has apparently not been explored, might be in screening known cell responses to stimulation in an attempt to detect those which show the behaviour to be expected of reactions intrinsic to the amplification stages of receptor mechanisms. We have chosen the specific example of the muscarinic cholinergic receptor to illustrate this suggestion, but the general method should be of wide applicability and the conclusions drawn below for the muscarinic cholinergic receptor may also be relevant to other receptors which control cell-surface Ca^{2+} permeability.

Various responses to stimulation of muscarinic cholinergic receptors are classified into five related groups in table 1, where the groups are arranged in an order which, from the information available, seems likely to mirror the sequence of events which occurs at the plasma membrane of a cell exposed to a muscarinic cholinergic agonist. For the sake of simplicity the responses are simply described in table 1 in terms of the concentrations of agonists needed to elicit an approximately half-maximal response, even though in some cases the dose–response curves deviate markedly from standard mass action curves (see later). In group 1 are events which provide direct assessments of the interaction between an agonist and its binding site on the receptor molecule. These, which will be discussed further in a later section, all show somewhat similar dose–response relationships, with half-saturation by carbamylcholine or acetylcholine in the approximate range 2–20 μM. From the considerations above, it therefore becomes clear that we are now interested in finding other responses which show dose–response curves of this general type. This immediately eliminates from consideration the 'physiological' responses in group 5 since they occur at much lower ('physiological') agonist concentrations. Also in group 5, and thus eliminated, is the elevation in intracellular concentration of cyclic GMP which has recently been under consideration as a putative second messenger in cholinergic responses [28]: not only does this occur at very low agonist concentrations in some responsive systems, but it also shows very different dose–response curves to cholinergic stimulation in different experimental systems [25–27]. The other three classes all show the required characteristic of a half-maximal response at relatively high agonist concentrations, so discrimination between these must draw upon other considerations. Efflux of Ca^{2+} from preloaded cells, the only event in group 3, is an expression of increased cell permeability to this ion. It tells us nothing of its cause, but is useful in that it provides confirmation of the type of dose–response curve to be expected of a reaction involved in producing this increased permeability. In group 4 are three events, namely cell K^+ (or Rb^+) permeability, membrane conductance and membrane depolarisation, which are probably all consequences of a change in cell-surface permeability to monovalent cations, K^+ in particular [24]. It appears likely from studies involving Ca^{2+} depletion [29], blockade by local anaesthetics [30] and the use of a Ca^{2+} ionophore to elevate intracellular Ca^{2+} [31] that these phenomena, like those in class 5, are, at least in part, secondary to the increase in intracellular Ca^{2+} concentration in stimulated cells.

The event which remains is phosphatidylinositol breakdown, the one member of group 2. This is characterised by both a high dose–response curve [17–21] and an independence of extracellular Ca^{2+} [32–34], suggesting that it is not a consequence of Ca^{2+} influx into stimulated cells. This is confirmed by the inability of Ca^{2+} introduced into cells with an ionophore to mimic this muscarinic cholinergic response [32,34] and by the inability of a variety of organic 'calcium antagonists' to prevent the phosphatidylinositol response to muscarinic cholinergic stimuli [35]. In addition, the phosphatidylinositol response is widespread and it seems likely that it occurs whenever any tissue which possesses muscarinic cholinergic receptors is exposed to high concentrations of an agonist [21,36].

Phosphatidylinositol breakdown (and secondarily its resynthesis) is therefore the only event which, when triggered by the muscarinic receptor, shows the characteristics expected of an event that is closely coupled to the activated receptor and which might therefore be involved in an essential amplification stage in the receptor system. This relationship is explored further in fig.1, which compares the only two available dose-response curves for the cholinergic phosphatidylinositol response with receptor occupation curves obtained with the same agonists: the latter are derived from studies of the inhibition of binding of propylbenzilylcholine mustard, a specific and irrever-

Table 1
The effects of agonist concentration on responses to stimulation of muscarinic cholinergic receptors

Class of response	Response	Tissue	Agonist	Concentration for half-maximal effects (unless otherwise specified)	Reference
Class 1: high dose-response, related to agonist-receptor interaction.	Calculated agonist-receptor interaction curve derived from dose-ratio studies of dibenamine-treated tissue.	Stomach fundus muscle	Carbamoylcholine	∿ 20 μM	[11]
	Competitive inhibition of reversible binding of quinuclidyl benzilate to receptor sites.	Ileum smooth muscle	Acetylcholine	∿ 2–4 μM	[12]
	Competitive inhibition of irreversible binding of propyl-benzilylcholine mustard to receptor sites.	Ileum smooth muscle	Carbamoylcholine	∿ 20 μM	[6,13–16]
Class 2: high dose-response, Ca^{2+}-independent.	Phosphatidylinositol breakdown, measured directly or detected through increased labelling.	Pancreas (labelling)	Acetylcholine	∿ 10 μM	[17–21]
		Pancreas (breakdown)	Acetylcholine	∿ 5–10 μM	
		Parotid gland (breakdown)	Acetylcholine	100 μM (very approx)	
		Ileum smooth smooth (labelling)	Carbamoylcholine	∿ 10 μM	
Class 3: high-dose response, a measure of Ca^{2+} permeability.	^{45}Ca efflux from preloaded tissue.	Isolated pancreatic acinar cells	Carbamoylcholine	∿ 50 μM	[22]
Class 4: high or moderate dose-response, mediated by Ca^{2+}.	K^+ (or Rb^+) efflux from preloaded tissue.	Ileum smooth muscle	Carbamoylcholine	∿ 30 μM	[23]
	Electrical conductance of cell surface membrane.	Ileum smooth muscle	Carbamoylcholine	Rises rapidly at > 1 μM, maximum not attained by ∿ 50 μM	[24]
	Depolarisation.	Ileum smooth muscle	Carbamoylcholine	∿ 1 μM (but see ref. [15])	[24]
Class 5: low dose-response, examples of 'physiological' responses mediated by Ca^{2+}.	Elevation of tissue cyclic GMP concentration.	Ileum smooth muscle	Carbamoyl-β-methylcholine	Maximal at 0.1 μM	[25]
		Neuroblastoma	Carbamoylcholine	∿ 0.01 nM	[26]
		Neuroblastoma	Carbamoylcholine	∿ 100 μM	[27]
	Protein secretion.	Pancreas	Acetylcholine	Maximal at 0.1 μM	[17]
	Contraction.	Ileum smooth muscle or stomach fundus muscle	Carbamoylcholine	∿ 0.1 μM	[11,23]

sible muscarinic antagonist, to the muscarinic receptor [6,13–16]. It should be noted that the methods used for obtaining the two dose–response curves for the phosphatidylinositol response differed considerably: in fig.1a the quantity recorded was the incorporation of $^{32}P_i$ in 30 min into the phosphatidylinositol of fragments of guinea pig ileum smooth muscle incubated with carbamoylcholine [20], whereas in fig.1b the assays were of the loss in 60 min of [^{3}H]inositol from the phosphatidylinositol of prelabelled mouse pancreases incubated with acetylcholine [18]. Despite these differences in tissue and assay procedure it is apparent that, at least for acetylcholine and carbamoylcholine, the dose–response curves for the activation of phosphatidylinositol metabolism are remarkably similar to the receptor occupation curves. In particular, the phosphatidylinositol response curves resemble the receptor occupation curves in being markedly flattened relative to normal mass-action curves: the explanation of this is uncertain, but might involve either some form of negative cooperativity or the existence of two receptor sub-populations with different affinities for agonists (see ref. [6]). The further prediction that for any chosen agonist the dose–response curve of the phosphatidylinositol response should be the same in all tissues cannot yet be tested precisely, but a review of the literature has already suggested that cholinergic phosphatidylinositol responses always require high agonist concentrations and that these concentrations always fall in approximately the same range [21].

Thus the phosphatidylinositol breakdown which is provoked by muscarinic cholinergic stimulation shows three key characteristics which might be expected of a reaction involved in bringing about increased cell-surface Ca^{2+} permeability in the stimulated cells and which are not exhibited by any other known response: (a) it shows a dose–response curve which is not typical for a biochemical response, but which closely follows the agonist-receptor interaction curve, (b) it is independent of changes in the intracellular Ca^{2+} concentration, and (c) it appears likely to be a universal response of all target cells. The idea that an enzyme reaction might be implicated in the mechanism of action of the muscarinic receptor is also favoured by the relatively long period of latency which intervenes between activation of muscarinic receptors and the detection of cell responses [37]. Furthermore, the occurrence of the phosphatidylinositol response in tissues exposed to other stimuli which increase cell-surface Ca^{2+} permeability, among them 5-hydroxytryptamine [38], α-adrenergic [39–41] and H_1-histaminergic [38], emphasises its possible close relationship to some general mechanism for the opening of receptor-controlled cell-surface Ca^{2+} gates [21,33,35].

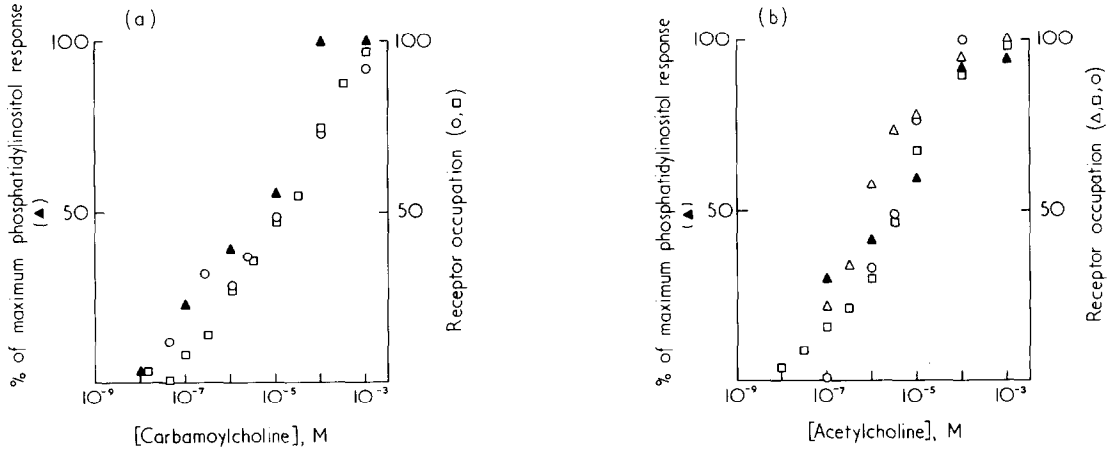

Fig.1. Comparison of dose–response curves for the phosphatidylinositol response and for muscarinic cholinergic receptor occupation by carbamoylcholine and acetylcholine. The methods used are discussed in the text. Filled triangles denote the phosphatidylinositol response, open symbols receptor occupation measurements. Figures were calculated using information from the following references: fig.1a (carbamoylcholine), ▲ (20), o (14), □ (15); fig.1b (acetylcholine), ▲ (18), o (13), □ (6), △ (16).

Acknowledgements

We are grateful to the Medical Research Council for financial support and to Dr David Allan for constructive criticism.

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