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SHORT COMMUNICATIONS

Stimulation of phosphatidylinositol turnover by histamine, 5-hydroxytryptamine and adrenaline in the longitudinal smooth muscle of guinea pig ileum

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The increase in phosphatidylinositol turnover which is elicited by physiological stimuli in various tissues has been studied for many years [1]. Recently it was suggested that breakdown of phosphatidylinositol, the reaction which initiates this increased turnover, may have a direct role in the sequence of events which links the agonist-receptor interaction to the final response of the cell [1, 2]: it was suggested that this reaction might be involved in the mechanisms of various receptor systems which increase the permeability of cell surfaces to Ca^{2+}.

It is thought that those agonists which stimulate contraction of the longitudinal smooth muscle of guinea pig ileum do so by elevating the intracellular concentration of Ca^{2+} ions. In this tissue, muscarinic cholinergic stimuli, which elicit contraction, cause a rapid increase in turnover of phosphatidylinositol [3]. Contraction can also be provoked by other stimuli, including histamine (acting through H_1 receptors), 5-hydroxytryptamine and, to a lesser extent, adrenergic stimuli interacting with α-excitatory receptors [4]. We have therefore investigated the effects of these stimuli on phosphatidylinositol metabolism and have concluded that each of the stimuli which provokes contraction also elicits, through the same receptor population, an increase in phosphatidylinositol turnover.

Materials and methods

Histamine, 5-hydroxytryptamine, carbamylcholine, adrenaline and mepyramine were from Sigma (London) Chemical Co. Ltd. or from sources previously specified [3, 5, 6]. Metiamide was from Smith, Kline & French, Welwyn Garden City, Herts, U.K. and methysergide bimaleate from Sandoz Ltd., Basle, Switzerland.

The methods used for tissue isolation, incubation with ^{32}Pi and lipid analysis were the same as were described previously [3]. As in the previous study, the experimental design was: (a) to incubate tissue fragments with ^{32}Pi for 30 min to label intracellular pools of ATP and other phospholipid precursors, and then (b) to add an agonist and continue incubation for a further 30 min. When antagonists were included they were normally present during both periods of incubation. Lipids were extracted and the separated phosphatidylinositol was analysed for phosphate and radioactivity [3].

Results

In the initial experiments, adrenaline, histamine and 5-hydroxytryptamine were tested at a concentration of 125 μM. Each produced a marked increase in phosphatidylinositol labelling, the effect produced by noradrenaline being about half that of the other agonists. We therefore proceeded to a study of the characteristics of the receptors responsible for these effects.

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The longitudinal smooth muscle of guinea pig ileum contains histamine receptors of both H_1 and H_2 types, with the former being responsible for the contractile response to histamine [7]. The increased phosphatidylinositol labelling produced by histamine was abolished by 12.5 μM mepyramine, an antagonist of H_1 receptors, but was unchanged by metiamide, which blocks H_2 receptors [8]. Table 1 also shows that neither mepyramine nor metiamide alone had any appreciable effect on phospholipid labelling. 12.5 μM mepyramine had no effect on the muscarinic cholinergic response to carbamylcholine.

The smooth muscle preparation contains muscle cells and nervous elements, both of which are sensitive to 5-hydroxytryptamine. The receptors in the nerve cells are designated M-receptors and are antagonised by morphine [4], whereas the receptors on the muscle cells, which directly trigger contraction, are methysergide-sensitive D-receptors.

Table 1. Effect of agonists and antagonists on phosphatidylinositol turnover

	Specific radioactivity of phosphatidylinositol (dmp ^{32}P per nmole)	Increase over control (%)
Experiment A		
No additions	81 ± 4(4)	—
Histamine (125 μM)	143 ± 2(3)	77
Mepyramine (12.5 μM)	87 ± 4(4)	7
Histamine (125 μM) + mepyramine (12.5 μM)	96 ± 5(5)	18
Metiamide (12.5 μM)	80 ± 7(5)	−1
Histamine (125 μM) + metiamide (12.5 μM)	142 ± 5(5)	75
Experiment B		
No additions	113	—
5-hydroxytryptamine (125 μM)	219	94
Carbamylcholine (125 μM)	236	109
Methysergide (10 μg/ml)	92	−19
5-hydroxytryptamine (125 μM) + methysergide (10 μg/ml)	108	−4
Carbamylcholine (125 μM) + methysergide (10 μg/ml)	235	108

Tissue fragments were incubated for 30 min with ^{32}Pi and for a further 30 min with ^{32}Pi and agonist (histamine, 5-hydroxytrypatmine or carbamylcholine). Antagonists (mepyramine, metiamide or methysergide) were present during both periods of incubation. Results in experiment A are mean ± S.E.M. (number of incubations) and in experiment B are mean values of duplicate (5-hydroxytryptamine) or triplicate (all others) incubations.

The D-receptors appeared to be responsible for the increased phosphatidylinositol labelling in response to 5-hydroxytryptamine since this response was abolished by methysergide bimaleate. This effect was specific in 5-hydroxytryptamine receptors since methysergide did not inhibit the response to carbamylcholine.

Adrenaline regularly produced a smaller stimulation of phosphatidylinositol labelling. In this tissue, adrenergic control is mainly inhibitory to contraction, but a distinct population of α-excitatory receptors is present [4]. Adrenergic control of phosphatidylinositol labelling in several tissues [including smooth muscle of rat vas deferens [9], rabbit iris [10] and cat aorta (E. G. Lapetina and P. Briley, personal communication)] is through α-adrenergic receptors [1, 2]. This also appears to apply to ileum smooth muscle, since its response was reproduced by phenylephrine, a specific α-agonist (S.S.J. and R.H.M., unpublished data). However, in view of the better systems available for the characterisation of α-adrenergic responses this effect was not explored in detail.

Discussion

Many tissues, including ileum smooth muscle, show increased phosphatidylinositol turnover when exposed to muscarinic cholinergic or α-adrenergic stimulation [1, 3, 9, 10, 11, 12]. Recently it was suggested that phosphatidylinositol breakdown, the reaction which is controlled by stimulation of receptors, might play an essential part in the mechanisms of action of these and other receptors which increase cell-surface permeability to Ca^{2+} [1, 2]. The effects of histamine and 5-hydroxytryptamine reported here appear to add support to this idea, since in both cases the receptors which increase intracellular Ca^{2+}, and thus provoke contraction, and those which stimulate phosphatidylinositol turnover appear to belong to the same pharmacological classes: the histamine receptors are of the mepyramine-sensitive H_1-type and the 5-hydroxytryptamine receptors are sensitive to inhibition by methysergide.

In earlier studies a less well-defined effect of histamine on phospholipid metabolism was observed in gastric mucosa [13] and, more recently, a small stimulatory effect of histamine on phosphatidylinositol metabolism has been observed in rat brain *in vivo* [14]. The latter response, like that of ileum smooth muscle, was sensitive to blockade of H_1 receptors, this time by tripelennamine [14]. Effects of 5-hydroxytryptamine on lipid metabolism have been studied more frequently, and stimulation of phosphatidylinositol metabolism has been observed in cerebral cortex [15, 16] and pineal gland [17, 18]. However, the present study appears to be the first in which it has been shown that specific 5-hydroxytryptamine receptors are involved.

It was noted previously that most receptors which trigger a phosphatidylinositol response also cause elevation of the intracellular concentrations of Ca^{2+} and of guanosine cyclic phosphate, and that receptors which stimulate adenylate cyclase do not usually have an obvious role in control of phosphatidylinositol metabolism [1]. The information reported here fits into this pattern, in that both 5-hydroxytryptamine and histamine (acting through H_1 receptors) stimulate phosphatidylinositol turnover and also elevate cellular Ca^{2+} and guanosine cyclic phosphate levels [19, 20]. In contrast, histamine probably acts on H_2-receptors to stimulate adenylate cyclase [21] and our information suggests no role for these receptors in control of phosphatidylinositol metabolism.

An observation consistent with the view that stimulated phosphatidylinositol breakdown might be integral to receptor mechanisms, rather than a typical cellular response produced by elevation of intracellular $[Ca^{2+}]$, is its complete or partial insensitivity to reduction of extracellular $[Ca^{2+}]$ in a variety of cells [1, 2, 11, 12, 22–24]. Preliminary studies of stimulated ileum smooth muscle suggest that it also shows increased phosphatidylinositol turnover in a Ca^{2+}-free medium or in the presence of some 'calcium antagonists' (S.S.J. and R.H.M., unpublished work), and it seems likely that this tissue will prove valuable in exploring the suggested relationship between phosphatidylinositol breakdown and receptor-controlled changes in cell surface permeability to Ca^{2+}.

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