Effects of Calcium-Antagonistic Drugs on the Stimulation by Carbamoylcholine and Histamine of Phosphatidylinositol Turnover in Longitudinal Smooth Muscle of Guinea-Pig Ileum

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A number of drugs classed as calcium antagonists, spasmolytics, non-specific receptor antagonists or receptor antagonists with multiple sites of action were tested to determine whether they prevent the stimulation of phosphatidylinositol turnover caused in various tissues by the activation of receptors which increase cell-surface Ca^{2+} permeability. The experiments were done with fragments of longitudinal smooth muscle from guinea-pig ileum; these were incubated *in vitro* with $^{32}\text{P}_i$ and either $100\mu\text{M}$ -carbamoylcholine or $100\mu\text{M}$ -histamine, in the presence of antagonistic drugs at concentrations at least sufficient to cause complete blockade of smooth-muscle contraction. The phosphatidylinositol response to carbamoylcholine was not changed by cinchocaine, papaverine, nifedipine, dibenamine, amethocaine, cinnarizine, lidoflazine, methoxyverapamil, prenylamine or two antimuscarinic alkane-bis-ammonium compounds, and the response to histamine was unaffected by the first four drugs. In contrast, phenoxybenzamine prevented the increase in phosphatidylinositol labelling caused by either carbamoylcholine or histamine. The insensitivity of the phosphatidylinositol response to most of the drugs provides further experimental support for the conclusion that the receptor-stimulated phosphatidylinositol breakdown which initiates the increase in phosphatidylinositol turnover is not caused by an increase in intracellular Ca^{2+} . The simplest interpretation of the available information appears to be that phosphatidylinositol breakdown plays a role in the coupling between the receptor-agonist interaction and the opening of cell-surface Ca^{2+} gates [Michell, R. H. (1975) *Biochim. Biophys. Acta* **415**, 81–147]. If this is correct, then phenoxybenzamine must exert its inhibitory effects on phosphatidylinositol breakdown early in this sequence of events, but the other drugs must act at a stage later than phosphatidylinositol breakdown. The unexpected difference in the effects of dibenamine and phenoxybenzamine, which are chemically very similar, may provide a useful experimental tool with which to explore the way in which activated receptors provoke the opening of cell-surface Ca^{2+} gates.

An effect brought about by activation of a variety of cell-surface receptors or by membrane depolarization in a variety of cells is an increase in cell-surface Ca^{2+} permeability (Hurwitz & Suria, 1971; Triggles, 1972; Douglas, 1974; Rubin, 1974; Berridge, 1975). The receptors that produce this effect include those that respond to 5-hydroxytryptamine and to muscarinic cholinergic, α -adren-ergic and H_1 -histaminergic stimuli. The resulting increase in the intracellular concentration of Ca^{2+} appears to be the major effector whereby these stimuli bring about responses such as secretion or contraction in their target cells. Consequently, these responses can be prevented by a variety of drugs ('non-specific receptor antagonists' or 'calcium antagonists') that are capable of blocking the receptor-provoked increase in cell-surface Ca^{2+} permeability: these drugs include phenoxybenzamine, dibenamine, papaverine, cinchocaine, amethocaine,

verapamil, methoxyverapamil (D600), cinnarizine, lidoflazine, nifedipine and prenylamine (Triggles, 1972; Godfraind & Kaba, 1972; Reuter, 1973; Fleckenstein *et al.*, 1975). There is also evidence that dibenamine, phenoxybenzamine and certain antimuscarinic alkane-bis-ammonium compounds might act at sites that are within the receptor system but distinct from the agonist-binding sites (Lüllman *et al.*, 1969; Triggles, 1971, pp. 298–303; Mitchelson, 1975). As a result, it seems likely that these drugs will continue to be useful as tools for investigating the molecular mechanism of this widespread receptor-controlled Ca^{2+} -gating system.

Stimulation of the same receptors also causes breakdown of phosphatidylinositol, and secondarily its resynthesis, in many or all target tissues (Michell, 1975; Michell *et al.*, 1976; Jafferji & Michell, 1976a,b). This response is atypical in that it does not appear to be a consequence of a rise in intracellular

[Ca²⁺] and this has been a major item of evidence for the view that it might play a role in the opening of cell-surface Ca²⁺ gates (Michell, 1975; Michell *et al.*, 1976*a,b*). We have therefore studied the effects of Ca²⁺ antagonists on this response in the hope that this might yield some further information on the interrelationship between receptors, Ca²⁺ gates and phosphatidylinositol metabolism.

Materials and Methods

Materials

Several reagents were from the same sources as in previous studies: phenoxybenzamine (*N*-phenoxyisopropyl-*N*-benzyl-2-chloroethylamine) (Michell & Jones, 1974)]; carbamoylcholine (Jafferji & Michell, 1976*a*); histamine (Jafferji & Michell, 1976*b*); cinchocaine [2-butoxy-*N*-(2-diethylaminoethyl)cinchoninamide] and amethocaine [*p*-butylaminobenzoyl-2-dimethylaminoethanol] (Allan & Michell, 1975). Papaverine (6,7-dimethoxy-1-veratrylisoquinoline) was from Sigma (London) Chemical Co., Kingston-upon-Thames, Surrey, U.K.; dibenamine (*NN*-dibenzyl-2-chloroethylamine) was from Smith, Kline and French, Welwyn Garden City, Herts., U.K.; cinnarizine (1-benzhydryl-4-cinnamylpiperazine) and lidoflazine {4-[4,4-bis-(4-fluorophenyl)butyl]-*N*-(2,6-dimethylphenyl)-1-piperazineacetamide} were from Janssen Pharmaceutica, Beerse, Belgium; nifedipine [4-(2'-nitrophenyl)-2,6-dimethyl-3,5-dicarboxymethoxy-1,4-dihydropyridine (Bay 1040)] was from Bayer U.K. Ltd., Pharmaceutical Division, Haywards Heath, West Sussex RH16 1TP, U.K.; and prenylamine (*N*-[3-phenylpropyl-(2)]-1,1-diphenylpropyl-3-aminolactate) was from Hoechst Pharmaceutical Research, Walton Manor, Milton Keynes, Bucks., MK7 8AJ, U.K. Methoxyverapamil (D600; α -isopropyl- α -(*N*-methyl-*N*-homoveratryl)-7-amino-propyl-3,4,5-trimethoxyphenylacetone nitrile) was a gift from Professor P. F. Baker, Department of Physiology, King's College, London. Heptane-1,7-bis(dimethyl-3'-phthalimidopropylammonium bromide) and the homologous hexane-1,6- compound were gifts from Dr. O. Wasserman, Institut für Pharmakologie, Universität Kiel, Kiel, West Germany.

Methods

The methods used for measurement of phosphatidylinositol metabolism were the same as before (Jafferji & Michell, 1976*a,b*). Briefly, the experimental design was to incubate fragments of guinea-pig ileum smooth muscle for 30 min in ³²P-labelled medium, containing a drug when necessary, and then to add either carbamoylcholine (100 μ M) or histamine (100 μ M) and continue the incubations for a further 30 min. The lipids were then extracted

and the specific radioactivity of phosphatidylinositol was determined. In each experiment the mean specific radioactivity of the control incubations was normalized to 100 and other experimental situations were compared with this: this allowed ready comparison and averaging of a large number of experiments. Table 1 shows a typical example of an experiment of the type averaged to produce the values in Tables 2 and 3. The effects of the drugs on phosphatidylinositol turnover in unstimulated and in carbamoylcholine-stimulated (or histamine-stimulated) tissues were assessed by applying an unpaired *t* test separately to the data from each individual experiment (see footnotes to Tables 2 and 3). The means of the responses observed in all experiments are presented in those Tables, together with standard deviations from three or more experiments or individual values when only two experiments were done.

Experiments with nifedipine were conducted in sodium light, since this compound is rapidly destroyed by u.v. light.

Results

Effects of antagonists on cholinergically stimulated phosphatidylinositol labelling

The tissue fragments were labelled with ³²P in the presence of various calcium antagonists at concentrations that have been reported to block smooth-muscle contraction effectively (see references cited in Table 2). Carbamoylcholine was then added and incubation was continued for a further period, after which incubations were terminated and the

Table 1. *Lack of effect of nifedipine on the stimulation of phosphatidylinositol turnover by carbamoylcholine*

Experimental details were as described in the Materials and Methods section. This is a typical experiment from those that were averaged to yield Tables 2 and 3. Results are means $\pm$ S.D. (numbers of incubations).

	Specific radioactivity of phosphatidylinositol (d.p.m./nmol)
No additions	62 $\pm$ 2 (4)
20 μ M-Nifedipine	64 $\pm$ 4 (3)*
100 μ M-Carbamoylcholine	144 $\pm$ 14 (3)†
100 μ M-Carbamoylcholine and 2 μ M-nifedipine	149 $\pm$ 19 (4)†
100 μ M-Carbamoylcholine and 20 μ M-nifedipine	148 $\pm$ 5 (3)*†

* Not significantly different from equivalent incubations without nifedipine.

† Significantly different from equivalent incubations without carbamoylcholine ($P < 0.005$).

Table 2. *Effects of drugs on carbamoylcholine-stimulated phosphatidylinositol labelling in fragments of guinea-pig ileum smooth muscle*

Incubation conditions were as described in the Materials and Methods section. Two to four replicate incubations were performed in each experiment and under each set of conditions. The mean value of the control from each experiment was then set at 100 and the other values were calculated as a percentage of this. Values for the other incubation conditions were then averaged from two to seven independent experiments, and either individual values or means $\pm$ s.d. are presented. The effects of drugs on the response to carbamoylcholine were assessed by statistical analysis of the data from each individual experiment by using an unpaired *t* test (see footnotes).

Antagonist (concentrations)	Specific radioactivity of phosphatidylinositol (% of control)			Effect on phosphatidylinositol response	Effect on contraction (Reference)
	+Antagonist	+Carbamoylcholine (100 μ M)	+Carbamoylcholine (100 μ M) + antagonist		
Cinchocaine (12.5 μ M)	111 (96, 126)	232 (144, 322)	230 (156, 304)	None†	Complete blockade (Luciani & Furlanut, 1974)
Amethocaine (62.6 μ M)	105 (109, 101)	259 (268, 250)	246 (207, 285)	None†	Complete blockade (Fleisch & Elwood, 1973)
Papaverine (12.5 μ M)	78 (81, 75)	199 $\pm$ 36 (4)	207 $\pm$ 46 (4)	None†	Complete blockade (Ferrari, 1974)
Methoxyverapamil (D600) (16 μ M)	82 $\pm$ 7 (4)	192 $\pm$ 26 (4)	206 $\pm$ 47 (4)	None†	Complete blockade (Fleckenstein <i>et al.</i> , 1975; Ticku & Triggle, 1976)
Cinnarizine (12.5–125 μ M)	94 $\pm$ 18 (3)	210 $\pm$ 31 (7)	220 $\pm$ 27 (7)	None†	Complete blockade (Godfraind & Kaba, 1972; Van Nueten & Janssen, 1973; Godfraind <i>et al.</i> , 1973)
Lidoflazine (50 μ M)	109 (116, 102)	196 (203, 184)	197 (226, 168)	None†	Complete blockade (Godfraind & Kaba, 1972)
'C _{7/3} -phthalimidopropyl'* (10 μ M)	98 (97, 98)	210 (218, 202)	212 (233, 189)	None†	Complete blockade (Lüllman <i>et al.</i> , 1969; Mitchelson, 1975)
'C _{6/3} -phthalimidopropyl'* (10 μ M)	84 (82, 86)	210 (218, 202)	193 (203, 182)	None†	Complete blockade (Lüllman <i>et al.</i> , 1969; Mitchelson, 1975)
Dibenamine (1–10 μ M)	99 $\pm$ 11 (4)	205 $\pm$ 34 (4)	201 $\pm$ 31 (4)	None†	Complete blockade (Triggle, 1971; p. 298)
Phenoxybenzamine (1.25–12.5 μ M)	85 (104, 66)	222 $\pm$ 53 (3)	116 $\pm$ 23 (3)	Complete blockade‡	Complete blockade (Triggle, 1971; p. 298)

* Heptane-1,7- (and hexane-1,6)-bis(dimethyl-3'-phthalimidopropylammonium bromide).

† Drugs did not cause a significant change in phosphatidylinositol labelling either in the absence or presence of carbamoylcholine ($P > 0.05$ in all experiments). In every case except one (in the absence of a drug) the increase in phosphatidylinositol labelling caused by carbamoylcholine was significant ($P < 0.05$) both in the absence and presence of added drugs; in about half of these estimates $P < 0.01$.

‡ Phenoxybenzamine alone had no effect on phosphatidylinositol labelling ($P > 0.05$), whereas carbamoylcholine stimulated it in the absence of phenoxybenzamine ($P < 0.05$) but not when the drug was present ($P > 0.05$).

labelling of phosphatidylinositol was measured. An example of the results from such an experiment is presented in Table 1. Carbamoylcholine (100 μ M) as a routine produced a doubling of the specific radioactivity of phosphatidylinositol under these conditions (Table 2), as has been observed previously (Jafferji & Michell, 1976a); this represents a fourfold increase in the rate of 32 P incorporation into this lipid during the latter half of the 1 h

incubation period. The experimental results were examined to determine whether the drugs prevented this increase in phosphatidylinositol turnover evoked by carbamoylcholine. The results are given in Table 2.

Local anaesthetics. These drugs, typified by cinchocaine and amethocaine, appear to prevent contractile activity in smooth muscle, by both interfering with Ca^{2+} mobilization from membrane-bound sites

and preventing the receptor-stimulated increase in membrane permeability to Ca^{2+} (Rubin *et al.*, 1967; Triggle, 1971, p. 534; Douglas, 1974). Neither cinchocaine nor amethocaine inhibited the cholinergically stimulated increase in phosphatidylinositol turnover (Table 2).

Papaverine. This is a very intensively studied spasmolytic drug which inhibits contractile responses to a variety of stimuli, especially the maintained tonic contractions which depend on a continuous influx of Ca^{2+} across the plasma membrane (Chang & Triggle, 1973a,b; Simonis *et al.*, 1971). It did not block the cholinergic stimulation of phosphatidylinositol turnover (Table 2).

Methoxyverapamil (D600), nifedipine, cinnarizine and lidoflazine. These are all compounds that exert their inhibitory effects on cell responses by preventing the Ca^{2+} influx across the cell surface which is normally evoked by receptor activation. This conclusion is based mainly on two observations: (a) elevation of extracellular $[\text{Ca}^{2+}]$ competitively inhibits the decrease in cell responses caused by the drugs (Godfraind & Kaba, 1972; Fleckenstein *et al.*, 1975), and (b) the drugs inhibit the stimulated entry of $^{45}\text{Ca}^{2+}$ into the tissue (Godfraind & Kaba, 1972). None of the four antagonists listed prevented the increase in phosphatidylinositol turnover evoked by carbamoylcholine (Table 1 and 2). In addition, we tested prenylamine, another calcium antagonist of quite high potency (Fleckenstein *et al.*, 1975). Although this compound itself caused an appreciable increase in phosphatidylinositol labelling, probably because its amphiphilic cationic character will cause it to stimulate phosphatidylinositol biosynthesis *de novo* (Allan & Michell, 1975), it did not appear to prevent the response to carbamoylcholine (S. S. Jafferji & R. H. Michell, unpublished work).

Dibenamine and phenoxybenzamine. These two drugs are both 2-halogenoalkylamine derivatives which alkylate some site(s), probably carboxylate(s), on receptor systems and thus produce an essentially irreversible blockade of a variety of Ca^{2+} -mobilizing receptor systems (Furchgott, 1966; Triggle, 1965, 1971). In general, their chemical and pharmacological similarities have suggested that they probably exert their inhibitory effects on receptor systems in the same way. We were therefore surprised when we observed that dibenamine did not change the response of phosphatidylinositol metabolism to cholinergic stimulation, whereas phenoxybenzamine completely blocked this response (Table 2).

Antimuscarinic alkane-bis-ammonium compounds. The two alkane-bis-ammonium compounds that we tested (see Table 2) possess high antimuscarinic activity, and both Lüllman *et al.* (1969) and Mitchelson (1975) have provided evidence that they exert this antagonism at a second binding site different from that at which agonists and atropine-

like drugs act. The nature of this second site was not defined, but it seemed to us that it might be related to the muscarinically controlled Ca^{2+} gates. Neither of the drugs prevented the cholinergic phosphatidylinositol response (Table 2), thus again confirming the distinctions between these drugs and the classical atropine-like antimuscarinic drugs (Jafferji & Michell, 1976a).

Effects of calcium antagonists on the stimulation of phosphatidylinositol metabolism by histamine

The experimental approach was the same as with cholinergic stimulation, except that histamine was used: activated H_1 -histamine receptors mobilize Ca^{2+} and stimulate contraction and phosphatidylinositol metabolism (Triggle, 1971, p. 532; Jafferji & Michell, 1976b). A smaller, but representative, range of drugs was selected for these experiments to determine whether significant differences in drug effects between the histaminergic and cholinergic responses would be found. No such differences were found, in that cinchocaine, papaverine, nifedipine and dibenamine had no inhibitory effect on histamine-stimulated phosphatidylinositol turnover, whereas phenoxybenzamine abolished this response (Table 3).

Discussion

There is little doubt that a progressive rise in intracellular Ca^{2+} constitutes the main effector system whereby muscarinic cholinergic and H_1 -histaminergic receptors being about dose-dependent cell responses such as contraction or secretion. In most cells this increase in intracellular Ca^{2+} is caused by an influx of Ca^{2+} through cell-surface Ca^{2+} gates which open in response to receptor activation (see, for example, Hurwitz & Suria, 1971; Triggle, 1972; Chang & Triggle, 1973a,b; Douglas, 1974; Rubin, 1974). Since phosphatidylinositol breakdown shows dose-response curves for neurotransmitters which are displaced to much higher concentrations than those of 'physiological' responses such as secretion or contraction (Hokin, 1968; Michell *et al.*, 1976a,b), it must be triggered either by a rise in intracellular Ca^{2+} concentration to a value higher than is needed to elicit these physiological responses or through some mechanism which is independent of changes in the intracellular Ca^{2+} concentration. If the former view is correct, i.e. it is triggered by a large rise in intracellular Ca^{2+} , then it should be more susceptible to inhibition by procedures designed to prevent the receptor-stimulated rise in intracellular Ca^{2+} concentration than are the physiological responses. In experiments reported so far, this has not been the case. On the contrary, the phosphatidylinositol response to muscarinic cholinergic stimulation of either adrenal medulla or parotid gland does

Table 3. *Effects of drugs on histamine-stimulated phosphatidylinositol labelling in guinea-pig ileum smooth muscle*

Incubation conditions etc. and the presentation and statistical analysis of results are as in Table 2, except that histamine was substituted for carbamoylcholine.

Antagonist (concentration)	Specific radioactivity of phosphatidylinositol (% of control)			Effect on phosphatidylinositol response
	+Antagonist	+Histamine (100 μ M)	+Histamine (100 μ M) and antagonist	
Cinchocaine (12.5 μ M)	100 $\pm$ 24 (3)	186 $\pm$ 4 (3)	193 $\pm$ 20 (3)	None†
Papaverine (12.5 μ M)	112 (80, 145)	200 (151, 251)	237 (195, 279)	None†
Nifedipine (20 μ M)	124 (74, 175)		187 (144, 231)	
(2 μ M)		194 (182, 206)	203 (144, 262)	None†
Dibenzamine (1 μ M)	86 $\pm$ 5 (3)	197 $\pm$ 50 (3)	186 $\pm$ 29 (3)	None†
Phenoxybenzamine (1.25–12.5 μ M)	90 (104, 76)	162 $\pm$ 20 (3)	77 $\pm$ 13 (3)	Complete blockade‡

* Drugs did not cause a significant change in phosphatidylinositol labelling either in the absence or presence of histamine ($P > 0.05$ in all experiments). In every case the increase in phosphatidylinositol labelling caused by histamine was significant ($P < 0.05$) both in the absence and in the presence of added drugs; in about half of these estimates $P < 0.01$.

† Phenoxybenzamine alone had no effect on phosphatidylinositol labelling ($P > 0.05$), whereas histamine stimulated it in the absence of phenoxybenzamine ($P < 0.05$), but not when the drug was present ($P > 0.05$).

Scheme 1. *Phosphatidylinositol breakdown as part of a mechanism for controlling the opening of cell-surface Ca^{2+} gates*

not require the presence of extracellular Ca^{2+} (Trifaró, 1969; Jones & Michell, 1975; Oron *et al.*, 1975), even though extracellular Ca^{2+} is essential for the physiological responses of these tissues (Selinger *et al.*, 1973; Douglas, 1974; Rubin, 1974; Oron *et al.*, 1975). These differences suggested that phosphatidylinositol breakdown might be a reaction with a functional role in the opening of cell-surface Ca^{2+} gates (Michell, 1975; Michell *et al.*, 1976), possibly in a sequence of events such as that depicted in Scheme 1.

When guinea-pig ileum smooth muscle is exposed to a Ca^{2+} -free medium it loses its ability to contract in response to stimuli within about 10 min (Ticku & Triggle, 1976). When similar experiments were done to determine the Ca^{2+} -dependence of the increase in phosphatidylinositol labelling in carbamoylcholine-stimulated fragments of ileum smooth muscle, it was found that most of the response was still apparent after Ca^{2+} deprivation for about 10 min, but that it largely disappeared in tissue that was deprived of Ca^{2+} for 30 min or more (S. S. Jafferji & R. H. Michell, unpublished work). This result was again in accord with the view that stimulation of phosphatidylinositol metabolism was less sensitive to Ca^{2+} deprivation than were other cell responses, and this fact, taken together with its requirement for a high against concentration

(Jafferji & Michell, 1976a), indicated that it was not controlled by the intracellular Ca^{2+} concentration. However, the results were less clear than those obtained with either the adrenal medulla or parotid gland, and we therefore decided to use drugs as an alternative method for preventing the receptor-induced changes in intracellular $[\text{Ca}^{2+}]$. These drugs have the advantage that their use does not require the extracellular surface of the plasma membrane to be deprived of Ca^{2+} , an ion which may be necessary for certain cell-surface receptors to respond to agonists (see, for example, Case & Clausen, 1973).

A variety of drugs act at the plasma membrane to prevent cells from responding normally to agonists but do not appear to achieve this effect by interfering with the agonist-receptor interaction, and there is evidence that many of these compounds interfere with the operation of receptor-sensitive Ca^{2+} gates in the plasma membrane (Godfraind & Kaba, 1972; Triggle, 1972; Fleckenstein *et al.*, 1975; Ticku & Triggle, 1976), i.e. they act at stages 2, 3 or 4 of Scheme 1. This conclusion is based on evidence from three independent types of experiment, each of which have been performed with various tissues, namely (1) inhibition of electrophysiologically measured Ca^{2+} currents through plasma membranes (Baker, 1972; Kohlhardt, 1972; Baker *et al.*, 1973;

Reuter, 1973; Riemer *et al.*, 1974), (2) inhibition of $^{45}\text{Ca}^{2+}$ movements into or out of cells (Shibata *et al.*, 1968; Godfraind & Kaba, 1972; Banerjee, 1974; Ticku & Triggle, 1976) and (3) inhibition of Ca^{2+} -mediated cell responses such as smooth-muscle contraction (Godfraind & Kaba, 1972; Fleckenstein *et al.*, 1975; Ticku & Triggle, 1976). Each type of study has included demonstrations that the drugs and Ca^{2+} behave competitively and the studies have not revealed any common mechanism of action of all of these drugs other than antagonism to Ca^{2+} gating. The experiments of Ticku & Triggle (1976) are particularly pertinent to those reported here, since they also investigated the effects of methoxyverapamil and nifedipine on cholinergically stimulated responses of the longitudinal smooth muscle of guinea-pig ileum. Their experiments are therefore closely comparable with ours except that we had to use the high concentrations of cholinergic agonists that are needed for the phosphatidylinositol response. To compensate for this, we have used much higher concentrations of antagonists, especially nifedipine, that they required for inhibition of contractility, and we have also tested a wide variety of drugs. Of the 12 drugs tested, only phenoxybenzamine abolished the phosphatidylinositol response to these stimuli. None of the other drugs had any detectable effect on the occurrence or magnitude of the phosphatidylinositol responses to either stimulus (Tables 2 and 3). These results provide confirmation, by using a novel method, that muscarinic, cholinergic and H_1 -histaminergic receptors do not trigger phosphatidylinositol breakdown through a change in intracellular $[\text{Ca}^{2+}]$.

It is possible that phosphatidylinositol breakdown is an event on a pathway of receptor response which is quite separate from that which leads to elevation of intracellular $[\text{Ca}^{2+}]$ (Michell, 1975; Oron *et al.*, 1975), but there is no evidence for such a widespread Ca^{2+} -independent route to control of cell responses by the appropriate receptors. It is for this reason that we have adopted the working hypothesis depicted in Scheme 1, in which phosphatidylinositol breakdown is placed at an early stage on the main reaction pathway which links the activation of cell-surface receptors to a rise in intracellular $[\text{Ca}^{2+}]$ (Michell, 1975; Michell *et al.*, 1976a,b). At present this hypothesis still appears to be compatible with the known characteristics of the phosphatidylinositol response, and the insensitivity of this response to all but one of the drugs we tested is seen simply as a result of these drugs acting at some step subsequent to phosphatidylinositol breakdown (i.e. step 3 or 4 of Scheme 1). Although this was to be expected for many of the accepted calcium antagonists, it was not expected for the antimuscarinic alkane-bis-ammonium compounds. For these, the lack of inhibition of the phosphatidylinositol response confirms that they act at a

site other than the agonist-binding site (Lüllman *et al.*, 1969; Mitchelson, 1975) and also suggests that this site is at stage 3 or 4 of the receptor-response sequence. It might therefore be worth while to test whether their anti-muscarinic activity is specific; maybe they are more general calcium antagonists?

Phenoxybenzamine, the only drug that inhibited the stimulation of phosphatidylinositol turnover by muscarinic cholinergic and H_1 -histaminergic stimuli, is also known to be an inhibitor of the α -adrenergic response in several tissues (Michell & Jones, 1974; Abdel-Latif, 1974; Canessa de Scarnatti & Lapetina, 1974; Hauser *et al.*, 1974); thus it prevents the response to at least three classes of Ca^{2+} -mobilizing receptors. This would place its point of action at stage 2 of Scheme 1, between the activation of a receptor site by an agonist molecule and the breakdown of phosphatidylinositol. It therefore seems likely that phenoxybenzamine, which alkylates some site within the receptor system and thus brings about irreversible blockade (Triggle, 1971, pp. 288–308), may prove very valuable in future studies of the mechanism that couples receptor activation to phosphatidylinositol breakdown.

One of the most surprising findings of the present study, and one which obviously calls for further investigation, was that the effects of two very closely related 2-halogenoalkylamine drugs, namely phenoxybenzamine and dibenamine, were quite different, with dibenamine producing no blockade of the phosphatidylinositol response to muscarinic cholinergic (Table 2) or H_1 -histaminergic (Table 3) stimulation.

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References

- Abdel-Latif, A. A. (1974) *Life Sci.* **15**, 961–967
- Allan, D. & Michell, R. H. (1975) *Biochem. J.* **148**, 471–478
- Baker, P. F. (1972) *Prog. Biophys. Mol. Biol.* **24**, 177–223
- Baker, P. F., Meves, H. & Ridgway, E. B. (1973) *J. Physiol. (London)* **231**, 511–526
- Banerjee, A. K. (1974) *Arch. Int. Pharmacodyn.* **207**, 148–161
- Berridge, M. J. (1975) *Adv. Cyclic Nucleotide Res.* **6**, 1–98
- Canessa de Scarnatti, O. & Lapetina, E. G. (1974) *Biochim. Biophys. Acta* **360**, 298–305
- Case, R. M. & Clausen, T. (1973) *J. Physiol. (London)* **235**, 75–102
- Chang, K.-J. & Triggle, D. J. (1973a) *J. Theor. Biol.* **40**, 125–154
- Chang, K.-J. & Triggle, D. J. (1973b) *J. Theor. Biol.* **40**, 155–172

- Douglas, W. W. (1974) *Biochem. Soc. Symp.* **39**, 1–28
- Ferrari, M. (1974) *Pharmacol. Res. Commun.* **6**, 97–115
- Fleckenstein, A., Nakayama, K., Fleckenstein-Grun, G. & Byon, Y. K. (1975) in *Calcium Transport in Contraction and Secretion* (Carafoli, E., Clementi, E., Drabikowski, W. & Margreth, A., eds.), pp. 555–566, North-Holland, Amsterdam
- Fleisch, J. H. & Elwood, T. (1973) *J. Pharmacol. Exp. Ther.* **186**, 44–51
- Furchgott, R. F. (1966) *Adv. Drug Res.* **3**, 31–55
- Godfraind, T. & Kaba, A. (1972) *Arch. Int. Pharmacodyn.* **196**, 35–49
- Godfraind, T., Kaba, A. & Rojas, R. (1973) *Br. J. Pharmacol.* **49**, 164–165
- Hauser, G., Shein, H. & Eichberg, J. (1974) *Nature (London)* **252**, 482–484
- Hokin, M. R. (1968) *Arch. Biochem. Biophys.* **124**, 280–284
- Hurwitz, L. & Suria, A. (1971) *Annu. Rev. Pharmacol.* **11**, 303–326
- Jafferji, S. S. & Michell, R. H. (1976a) *Biochem. J.* **154**, 653–657
- Jafferji, S. S. & Michell, R. H. (1976b) *Biochem. Pharmacol.* **25**, 1429–1430
- Jones, L. M. & Michell, R. H. (1975) *Biochem. J.* **148**, 479–485
- Kohlhardt, M., Bauer, B., Krause, H. & Fleckenstein, A. (1972) *Pflügers Arch.* **335**, 309–322
- Luciani, S. & Furlanut, M. C. (1974) *Pharmacol. Res. Commun.* **6**, 142–153
- Lüllman, H., Ohnesorge, F. K., Schauwecker, G.-C. & Wasserman, O. (1969) *Eur. J. Pharmacol.* **6**, 241–247
- Michell, R. H. (1975) *Biochim. Biophys. Acta* **415**, 81–147
- Michell, R. H. & Jones, L. M. (1974) *Biochem. J.* **138**, 47–52
- Michell, R. H., Jones, L. M. & Jafferji, S. S. (1976a) in *Stimulus-Secretion Coupling in the Gastrointestinal Tract* (Case, R. M. & Goebbel, H., eds.), pp. 89–105, Medical and Technical Publishing, Lancaster
- Michell, R. H., Jafferji, S. S. & Jones, L. M. (1976b) *FEBS Lett.* in the press
- Mitchelson, F. (1975) *Eur. J. Pharmacol.* **33**, 237–246
- Oron, Y., Lowe, M. & Selinger, Z. (1975) *Mol. Pharmacol.* **11**, 79–86
- Reuter, H. (1973) *Prog. Biophys. Mol. Biol.* **26**, 1–43
- Riemer, J., Dörfler, F., Mayer, C.-J. & Ulbrecht, G. (1974) *Pflügers Arch.* **351**, 241–248
- Rubin, R. P. (1974) *Calcium and the Secretory Process*, Plenum Press, New York
- Rubin, R. P., Feinstein, M. B., Jaanus, S. P. & Paimre, M. (1967) *J. Pharmacol. Exp. Ther.* **155**, 463–471
- Selinger, Z., Batzri, S., Eimerl, S. & Schramm, M. (1973) *J. Biol. Chem.* **248**, 369–372
- Shibata, S., Carrier, O. & Frankenheim, J. (1968) *J. Pharmacol. Exp. Ther.* **160**, 106–111
- Simonis, A. M., Ariëns, E. J. & Van den Broeke, J. J. W. (1971) *J. Pharm. Pharmacol.* **23**, 107–110
- Ticku, M. K. & Triggle, D. J. (1976) *Gen. Pharmacol.* in the press
- Trifaró, J. (1969) *Mol. Pharmacol.* **4**, 424–427
- Triggle, D. J. (1965) *Adv. Drug Res.* **2**, 173–189
- Triggle, D. J. (1971) *Neurotransmitter-Receptor Interactions*, Academic Press, New York
- Triggle, D. J. (1972) *Adv. Surf. Membr. Sci.* **5**, 267–331
- Van Nueten, J. M. & Janssen, P. A. J. (1973) *Arch. Int. Pharmacodyn.* **204**, 37–55