Sympathetic denervation impairs agonist-stimulated phosphatidylinositol metabolism in rat parotid glands

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1. Substance P, muscarinic and α -adrenoceptor agonists stimulated the incorporation of [3 H]inositol into phosphatidylinositol in rat parotid gland slices. Surgical denervation of the sympathetic input to the rat parotid gland by superior cervical ganglionectomy produced marked reductions in these responses. 2. The stimulated incorporation of radiolabelled precursors into phosphatidylinositol is a measure of its resynthesis after receptor-mediated breakdown of inositol phospholipids. We therefore examined the enzymic site of the lesion induced by sympathetic denervation using parotid gland slices labelled with either [3 H]inositol or [32 P]phosphate and stimulated with substance P. 3. Receptor-activated phospholipase C attack upon [3 H]inositol phospholipids was assayed by measuring the formation of [3 H]inositol 1-phosphate in the presence of 10 mM-Li $^+$ to inhibit further breakdown. It was not affected by denervation. 4. Substance P elicited a rapid breakdown of phosphatidylinositol 4,5-bisphosphate and this response was reduced in the denervated gland. 5. The second step in stimulated phosphatidylinositol turnover, phosphorylation of diacylglycerol to phosphatidate was not affected by denervation. 6. Sympathetic denervation appears to induce a specific enzymic lesion in the parotid gland that impairs receptor-stimulated resynthesis of phosphatidylinositol from phosphatidate. This change in membrane lipid metabolism may be related to a number of the effects of sympathetic denervation, such as agonist supersensitivity, reduced gland cell proliferation and induction of new surface receptors.

A number of structural and functional changes occur after sympathetic denervation of the salivary glands (Wilborn & Schneyer, 1972; Ekstrom, 1980). There is a marked increase in the density but not the binding characteristics of post-synaptic α_2 -adrenoceptors and more modest increases in the densities of α_1 - and β_1 -adrenergic receptors (Bylund & Martinez, 1981; Bylund *et al.*, 1982). By contrast there is no change in the density of muscarinic

receptors. Concomitant with these receptor changes, there are functional changes in the sensitivity of the glands to autonomic agonists. As in other tissues (Fleming, 1981), surgical sympathectomy induces supersensitivity to sympathetic stimuli so that receptor-coupled events such as K $^+$ efflux and cyclic AMP formation are enhanced or are provoked by lower agonist concentrations (Bylund *et al.*, 1981, 1982). However, the enhancement of these functional responses does not appear to be simply linked to the appearance of new receptors, leading to the speculation that these secondary events are enhanced as a consequence of a fundamental change in the stimulus–response effector systems of the gland.

Two classes of events have been suggested to be involved in stimulus–response coupling in the parotid gland of the rat: regulation of cyclic nucleotides and an increase in the cytosol Ca $^{2+}$ concentration (Putney, 1979). One experimental approach has been to examine the effects of sympathectomy upon the enzymes of cyclic AMP metabolism and this has revealed a reduction in

Abbreviations used: PtdIns, phosphatidylinositol; KRB, Krebs–Ringer bicarbonate; PtdIns4P, phosphatidylinositol 4-phosphate; PtdIns(4,5)P $_2$, phosphatidylinositol 4,5-bisphosphate; PtdA, phosphatidic acid; Ins1P, inositol 1-phosphate.

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cyclic nucleotide phosphodiesterase activity. This observation may explain the enhanced cyclic AMP accumulation after receptor stimulation of the denervated gland (Bylund *et al.*, 1981).

It is now clear that the receptors on rat parotid glands for substance P, α -adrenoceptor and muscarinic ligands share a common mechanism of action that involves an increase in the cytosolic Ca^{2+} concentration (Ca^{2+} -mobilization). Activation of these receptors produces marked changes in inositol phospholipid metabolism. Thus when rat parotid gland fragments or isolated acinar cells are incubated with [^3H]inositol or [^{32}P]P_i and stimulated with Ca^{2+} -mobilizing agonists there is a large increase in the rate of radioisotope incorporation into PtdIns (Hokin & Sherwin, 1957; Jones & Michell, 1974; Oron *et al.*, 1975; Putney, 1979; Hanley *et al.*, 1980). The initial response is accelerated hydrolysis of inositol phospholipids, either of PtdIns and/or of polyphosphoinositides (Weiss *et al.*, 1982b). This generates diacylglycerol in the membrane, which is rapidly converted into PtdA (Weiss *et al.*, 1982a). The increase in PtdA concentration leads to enhanced resynthesis of PtdIns, which probably accounts for the increased incorporation of radioisotopes into this lipid.

The receptor-stimulated hydrolysis and subsequent resynthesis of inositol phospholipids is thought to have an integral role in the transduction process that leads to Ca^{2+} -mobilization (Michell *et al.*, 1981). We now report that sympathetic denervation of rat parotid glands profoundly alters the receptor-stimulated metabolism of these phospholipids.

Methods

Surgery

Surgical denervations of the sympathetic input to the parotid gland were made on 200–300 g male Sprague–Dawley rats under Equithesin anaesthesia, by unilateral removal of the superior cervical ganglion. The success of the operation was judged by the appearance of ptosis on the operated side. For sham operations, the bifurcation of the carotid artery was exposed. In all cases, glands from the unoperated side served as controls. As an additional control, a comparison between bilaterally sympathectomized animals and bilateral sham-operated animals revealed no significant difference from the use of unilateral lesions. Experiments on parotid glands were undertaken 10–14 days after surgery.

Tissue preparation and incubation

The dissection and preparation of rat parotid gland fragments has been described in detail previously (Hanley *et al.*, 1980). Portions (50 μl) of the gently packed fragments were pipetted into plastic vials (Beckman Bio-Vials) containing either *myo*-

[2- ^3H]inositol (New England Nuclear) or [^{32}P]-orthophosphate (Amersham) in a final volume of 250 μl of modified KRB buffer. For incubations with [^{32}P]P_i the concentration of KH_2PO_4 was reduced to 0.28 mM to give improved incorporation of labelled phosphate into parotid lipids. Drug additions (10 μl) were made from serially diluted stock solution in KRB buffer. Vials were gassed with O_2/CO_2 (19:1), capped and incubated at 37°C in a shaking water bath.

Phospholipid analysis

When parotid gland fragments were incubated with *myo*-[2- ^3H]inositol, radiolabelled phospholipids were extracted and counted for radioactivity as described previously (Hanley *et al.*, 1980).

When parotid gland fragments were incubated with [^{32}P]P_i (60 min at 37°C), radiolabelled lipids were determined by two procedures. Incubations were first stopped by the addition of 0.94 ml of chloroform/methanol/HCl (40:80:1, by vol.). Chloroform (0.31 ml) and 0.1 M-HCl (0.31 ml) were then added, and the samples briefly centrifuged to give two phases. The upper phase was discarded and the lower phase (including flocculent material at the phase interface) was washed once with its synthetic upper phase.

A portion of the washed lower phase was removed (50 μl), dried under N_2 and counted by scintillation counting to determine the total radioactivity in the phospholipid fraction.

To measure the polyphosphoinositides, a portion of the lower phase (0.45 ml) was dried under N_2 , redissolved in chloroform (0.5 ml) and deacylated by alkaline hydrolysis for subsequent separation of the glycerol derivatives of the polyphosphoinositides on anion-exchange columns, as described previously (Downes & Michell, 1981).

To separate the radiolabelled phospholipids, a portion of the lower phase (0.45 ml) was dried under N_2 , redissolved in 0.1 ml of chloroform/methanol/water (75:25:2, by vol.), and applied to high-performance t.l.c. plates (Merck Kieselgel 60) previously impregnated with oxalate (Jolles *et al.*, 1979). The plates were developed in chloroform/acetone/methanol/acetic acid/water (40:15:13:12:8, by vol.). Radiolabelled phospholipids were located by autoradiography overnight using Kodak X-Omat GR film. Phosphatidyl-inositol, PtdIns4P, PtdIns(4,5)P₂ and PtdA were identified by co-chromatography with commercial standards (Sigma). Areas of the plate corresponding in mobility to those of the standards were scraped off and counted for radioactivity by liquid-scintillation spectrometry. Comparison of the total radioactivity extracted from the plates in each lane with the corresponding portion of the original lower phase indicated recoveries of 75–90% from the

high-performance t.l.c. step. Recovery values were calculated for each individual lane and used as correction factors.

[³H]Ins1P analysis

For measurement of [³H]Ins1P release from inositol phospholipids, 10 mM-LiCl was included in the incubation to inhibit inositol 1-phosphatase (Sherman *et al.*, 1981). Basal and stimulated labelling of Ins1P were determined as described previously (Berridge *et al.*, 1982) except that 0.2 M-ammonium formate/0.005 M-disodium tetraborate was used to elute labelled compounds from Dowex-1 (formate form) anion-exchange resin rather than 1 M-ammonium formate in 0.1 M-formic acid. The only ³H-labelled compounds eluted from the resin under these conditions are glycerophosphoinositol (the deacylation product of PtdIns) and Ins1P (Berridge *et al.*, 1983). Stimulation of parotid gland slices with carbachol, phenylephrine or substance P in the presence of 10 mM-LiCl increases only the Ins1P fraction with no detectable change in labelled glycerophosphoinositol. Therefore this assay procedure is a specific measure of receptor-stimulated phospholipase C attack upon inositol phospholipids.

Results

Effect of ganglionectomy on receptor-stimulated [³H]inositol incorporation into PtdIns

Supramaximal doses of the α -agonist phenylephrine, the cholinergic agonist carbachol and substance P each provoked a similar stimulation of [³H]inositol incorporation into PtdIns in control parotid glands. The enhancement of radioisotope incorporation due to substance P was similar to that obtained in previous experiments (Hanley *et al.*, 1980). However, experiments with [³²P]_i-labelling of PtdIns and PtdA (not shown) and with [³H]inositol phosphate formation (Berridge *et al.*, 1983) agree with the previously published observation (Weiss & Putney, 1981) that muscarinic cholinergic ligands produce a much larger stimulation of inositol lipid metabolism than α -adrenergic ligands, which are in turn more efficacious than substance P. Furthermore, the enhanced incorporation of [³²P]_i into PtdIns is always more marked than enhanced incorporation of [³H]inositol (C. P. Downes, unpublished work), perhaps because inositol can be incorporated into PtdIns through a phospholipid head-group exchange mechanism that is not part of the cycle of reactions initiated by hormones and neurotransmitters (Yandrasitz & Segal, 1979). The precise reasons for these different observations arising from experiments using different radioisotopes remain obscure.

At 10 days after surgical sympathectomy this receptor-stimulated increase in the rate of [³H]-

inositol incorporation into PtdIns was virtually abolished (Table 1). This was not simply an effect of physical trauma since the receptor responses were unimpaired after sham operations. Furthermore the basal rate of incorporation of [³H]inositol into parotid gland phospholipids was the same in the control and denervated glands. Typical values for this incorporation in a 60 min incubation were 117000 ± 13800 d.p.m. per sample (mean $\pm$ S.E.M. for three incubations) for control glands compared with 120100 ± 6000 d.p.m. for the denervated glands.

Subsequent experiments were designed to identify the enzymic lesion(s) induced by denervation. In order to reduce the number of variables we focused on substance P as the receptor stimulant. This choice was important because we wished to concentrate on changes in the molecular events common to each of the Ca²⁺-mobilizing agonists that follow receptor occupation and the use of α -adrenoceptor agonists could have been confounded by changes in the number and sub-types of receptors after sympathectomy. Previous work suggests a close relationship between the substance P-elicited secretory response and stimulated PtdIns metabolism (Hanley *et al.*, 1980; Brown & Hanley, 1981).

Receptor-stimulated Ins1P formation

The most direct method for measuring receptor-stimulated phospholipase C attack upon inositol phospholipids is to detect the liberation of inositol phosphates. We recently reported that this can be done with high sensitivity if Li⁺ is included in the incubations to inhibit *myo*-inositol 1-phosphatase (Berridge *et al.*, 1982). The technique provides a novel means of determining small changes in receptor-coupled inositol phospholipid breakdown.

When parotid gland fragments were labelled with [³H]inositol for 60 min and then exposed to substance P (4 μ M) and lithium (10 mM) there was a rapid increase in the level of radioactivity found in the Ins1P fraction. This enhanced [³H]Ins1P formation was sustained for at least 15 min after the initial exposure to substance P (Table 2). However, there was no significant difference in the rates of [³H]Ins1P formation in the tissue fragments obtained from control and denervated parotid glands.

[³²P]_i-labelling experiments

By contrast with the [³H]inositol labelling experiments, surgical sympathectomy caused a marked reduction in the basal rate of [³²P]_i incorporation into parotid gland phospholipids. The magnitude of this reduction varied between experiments from 30 to 60% and was similar for each of the phospholipid fractions analysed [PtdIns, PtdIns4P, PtdIns-(4,5)P₂, PtdA and other labelled phospholipids combined]. Thus the proportion of the total radio-

Table 1. *Effect of superior cervical ganglionectomy on receptor-stimulated incorporation of [³H]inositol into phospholipids in rat parotid gland slices*

Slices were incubated with [³H]inositol (0.32 μ M, 12.5 Ci/mmol) for 30 min before the addition of drugs in order to equilibrate radiolabelled inositol with the intracellular pool. Supramaximal doses of peptidergic (substance P), α -adrenoceptor (phenylephrine) and muscarinic (carbachol) agonists were added for 30 min. Basal labelling (in the absence of added drug) was normalized to 100%. In individual experiments, results were the means of triplicate determinations. Results are expressed as means $\pm$ S.E.M. The number of individual experiments (*n*) is given in parentheses. *Significantly different from the unoperated gland at *P* < 0.01 (by paired *t*-test).

Stimulant	Stimulation as proportion of basal [³ H]inositol incorporation (%)			
	Sham ganglionectomy		Ganglionectomy	
	Control	Operated	Control	Operated
Phenylephrine (0.5 mM)	166	183 (<i>n</i> = 2)	172 $\pm$ 22	103 $\pm$ 4* (<i>n</i> = 3)
Substance P (4 μ M)	166 $\pm$ 2	153 $\pm$ 9 (<i>n</i> = 5)	172 $\pm$ 11	113 $\pm$ 5* (<i>n</i> = 14)
Carbachol (0.5 mM)	150	152 (<i>n</i> = 2)	152 $\pm$ 10	110 $\pm$ 8* (<i>n</i> = 6)

Table 2. *Receptor-stimulated inositol 1-phosphate formation in tissue slices from control and superior cervical ganglionectomized parotid glands*

Slices were incubated with [³H]inositol (0.32 μ M, 12.5 Ci $\cdot$ mm⁻¹) for 60 min to label the inositol phospholipids. LiCl (150 mM) was then added to a final concentration of 10 mM and the samples were incubated for a further 5 min. The slices were then exposed to substance P (4 μ M), for 5 or 15 min before determining the radioactivity in the [³H]Ins1P fraction. Results are means $\pm$ S.E.M. for three samples in a single experiment using tissues pooled from three animals. Three separate experiments gave similar results.

	Radioactivity (d.p.m.) in [³ H]Ins1P fraction			
	5 min stimulation		15 min stimulation	
	Basal	+ Substance P	Basal	+ Substance P
Unoperated	8560 $\pm$ 360	13 500 $\pm$ 410	8780 $\pm$ 630	21 100 $\pm$ 220
Ganglionectomy	7530 $\pm$ 490	13 770 $\pm$ 40	8320 $\pm$ 320	23 980 $\pm$ 480

activity found in each of the phospholipid fractions in both the normal and denervated glands was similar (Table 3). Since the incorporation of the radiolabel into each of these phospholipid species occurs at quite separate metabolic steps this suggests that a reduced specific radioactivity of the ATP pool, rather than a marked decrease in the concentration of this intermediate, is likely to account for the altered phospholipid labelling. The results from the operated and unoperated glands were compared by expressing the radioactivity in phospholipids from incubations with agonist as a percentage of that in phospholipids from control incubations. Thus for the unoperated glands, using conditions of stimulation identical with that for [³H]inositol incorporation (see the legend to Table 1), 4 μ M-substance P increased the radioactivity in ³²P-labelled PtdIns to 290 $\pm$ 50% (mean $\pm$ S.E.M. for three experiments) of the basal level. This value was markedly reduced to 142 $\pm$ 3% in ganglionectomized glands. Incubation of tissue fragments with [³²P]P_i and separation of the labelled products by high-performance t.l.c., therefore, confirmed the previous observation when the radiolabel was [³H]inositol.

Receptor-stimulated PtdIns(4,5)P₂ breakdown

In these experiments the tissue fragments were prelabelled with [³²P]P_i for 60 min and then exposed to substance P for 1 min before extraction and separation of phospholipids. The peptide evoked a 35–40% decrease in the radioactivity found in PtdIns(4,5)P₂ in several separate experiments on unoperated glands (Table 4a). This observation, and the fact that we could not detect any loss of radioactivity from PtdIns4P after brief stimulation, is very similar to that of Weiss *et al.* (1982b), who used isolated parotid acinar cells for their experiments. More importantly for the purpose of the present paper, the PtdIns(4,5)P₂ breakdown response to substance P was attenuated in the denervated gland (Table 4a).

Conversion of diacylglycerol into PtdA

The second step in the cycle of reactions postulated to be involved in receptor-stimulated PtdIns turnover is the phosphorylation of diacylglycerol to PtdA (see Fig. 1). This enzymic step was assessed in denervated and control glands using

Table 3. *The effects of denervation on the incorporation of [32 P] P_i into rat parotid gland phospholipids*

Slices were incubated with [32 P] P_i (10 μ Ci per incubation) for 60 min before determining the radioactivity in each of the phospholipid fractions after separation on high-performance t.l.c. The results are means $\pm$ S.E.M. for three incubations.

Phospholipid species	[32 P] P_i incorporation per incubation (d.p.m.)		Proportion of total (%)	
	Unoperated	Ganglionectomy	Unoperated	Ganglionectomy
PtdIns	46 080 $\pm$ 4440	24 210 $\pm$ 5050	34	36
PtdA	20 800 $\pm$ 780	8490 $\pm$ 560	15	13
PtdIns4P	10 660 $\pm$ 500	4070 $\pm$ 450	7.8	6.1
PtdIns(4,5) P_2	15 990 $\pm$ 850	6100 $\pm$ 980	12	9.1
Other phospholipids combined	43 300 $\pm$ 2560	24 260 $\pm$ 1980	32	36

Table 4. *Effect of superior cervical ganglionectomy on receptor-stimulated PtdIns(4,5) P_2 breakdown (a) and PtdA formation (b) in rat parotid slices*

Slices were incubated with [32 P] P_i (10 μ Ci per incubation) for 60 min. They were exposed to the stimulant, substance P (4 μ M), for 1 min and the radioactivity in PtdIns(4,5) P_2 or PtdA determined. Basal rates of incorporation (in the absence of any stimulus) were normalized to 100%. Results are means $\pm$ S.E.M. for the number of experiments (n) given in parentheses. *Statistically different from results for the unoperated gland at $P < 0.01$ (by paired t -test).

	Stimulated
(a) PtdIns(4,5) P_2 breakdown	
Unoperated	62 $\pm$ 2.7% ($n = 12$)
Ganglionectomy	81 $\pm$ 3.7 ($n = 10$)*
(b) PtdA formation	
Unoperated	176 $\pm$ 17% ($n = 3$)
Ganglionectomy	156 $\pm$ 2.5% ($n = 3$)

incubation conditions identical with those for determining PtdIns(4,5) P_2 breakdown. The results (Table 4b) are very similar to those obtained by Weiss *et al.* (1982a) using parotid acinar cells stimulated with substance P. There was no significant difference between the results obtained for denervated glands and those for control tissue.

Discussion

Nature of the biochemical lesion induced by sympathectomy

The most commonly used measure of receptor-stimulated PtdIns metabolism, the enhanced incorporation of [32 P] P_i or [3 H]inositol into PtdIns, was markedly reduced 10–14 days after superior cervical ganglionectomy. However, since these measurements of phospholipid resynthesis could have been indicative of a primary fault in the rapid events suggested to be closely coupled to receptor activation, these events were examined directly. Thus receptor-stimulated phospholipase C activity

and phosphatidate formation were not significantly affected by denervation and the lesion appears to be localized to the enzymic steps that convert PtdA into PtdIns. In addition, sympathetic denervation reduces the substance P-stimulated disappearance of PtdIns(4,5) P_2 . Thus denervation exerts distinct effects upon PtdIns resynthesis and PtdIns(4,5) P_2 disappearance.

The observation that receptor-stimulated phospholipid breakdown can occur without necessarily leading to enhanced radioisotope labelling of PtdIns is not new. A similar situation exists in the blowfly salivary gland stimulated with 5-hydroxytryptamine (Berridge & Fain, 1979), because Ca^{2+} entry into the glands inhibits PtdIns resynthesis. This suggests a plausible explanation for the effect of sympathectomy on receptor-stimulated PtdIns metabolism in the rat parotid gland. Thus denervation could lead to supersensitivity of the Ca^{2+} -mobilizing class of receptors, enhanced intracellular Ca^{2+} concentration during stimulation and feedback inhibition of PtdIns resynthesis.

Denervation perturbs receptor-stimulated polyphosphoinositide metabolism

Recent work suggests that polyphosphoinositide hydrolysis should replace PtdIns breakdown as the initiating reaction in receptor-stimulated inositol phospholipid metabolism (Michell *et al.*, 1981; Weiss *et al.*, 1982b; Creba *et al.*, 1983). This conclusion is strongly supported by the detection of [3 H]inositol-labelled inositol 1,4,5-trisphosphate and inositol 1,4-bisphosphate, which increase rapidly after stimulation of rat parotid glands, insect salivary glands or brain slices with Ca^{2+} -mobilizing agonists (Berridge *et al.*, 1983; C. P. Downes & M. Wusteman, unpublished work). Ins1P probably arises from hydrolysis of these inositol polyphosphates. However, it is difficult to reconcile this view with the observation that sympathetic denervation reduces substance P-stimulated PtdIns(4,5) P_2 disappearance without altering the rate of formation of [3 H]Ins1P. A possible

explanation for this apparent paradox is that the rate of polyphosphoinositide resynthesis (through PtdIns kinase and PtdIns4P kinase) is enhanced in the denervated glands. More importantly, the lack of effect of denervation on PtdA labelling and labelled inositol phosphate formation compared with its effects on stimulated PtdIns labelling and PtdIns-(4,5) P_2 disappearance should serve as an example of the difficulties in attempting precise interpretations of information from lipid labelling studies.

Inositol phospholipids and cellular responsiveness

An interesting possibility is that the changes in stimulated inositol phospholipid metabolism induced by sympathetic denervation may underly the changes in cellular responsiveness that result from this lesion. Recent evidence suggests that PtdIns may be closely associated with adenylate cyclase in heart sarcolemma (Panagia *et al.*, 1981) and turkey erythrocytes (Low & Finnean, 1977) and (Na⁺ + K⁺)-stimulated ATPase in membranes of a kidney microsomal preparation (Roelofsen & Van Linde-Sibenius Trip, 1981), two enzymes that may be important for determining the sensitivity of many cells to stimulation of their cell-surface receptors. Perhaps inositol phospholipid hydrolysis is coupled to receptor-stimulated Ca²⁺-mobilization as first suggested by Michell (1975), whereas the subsequent resynthesis of PtdIns may be involved in more subtle effects upon the overall sensitivity of the cell. In any case our results appear to demonstrate separable effects of receptor stimulation upon inositol phospholipid hydrolysis and upon their resynthesis from phosphatidate.

It is not clear to what extent stimulus-secretion coupling events may be related to long-term changes in glandular structure induced by changes in sympathetic receptor activity. However, it has been suggested elsewhere that in secretory glands a common biochemical mechanism may underly both of these processes (Pawlikowski, 1982). Altered inositol phospholipid metabolism may therefore have a role to play in long-term proliferative changes brought about by sympathetic denervation or chronic stimulation with sympathomimetics. Further studies on the effects of sympathectomy on receptor-stimulated inositol phospholipid metabolism in salivary glands may provide an insight into functional alterations in glandular activity and the trophic control of cellular functions by the sympathetic nervous system.

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References

- Berridge, M. J. & Fain, J. N. (1979) *Biochem. J.* **178**, 59–69
- Berridge, M. J., Downes, C. P. & Hanley, M. R. (1982) *Biochem. J.* **206**, 587–595
- Berridge, M. J., Dawson, R. M. C., Downes, C. P., Heslop, J. P. & Irvine, R. F. (1983) *Biochem. J.* **212**, 473–482
- Brown, C. L. & Hanley, M. R. (1981) *Br. J. Pharmacol.* **73**, 517–523
- Bylund, D. B. & Martinez, J. R. (1981) *J. Neurosci.* **1**, 1003–1007
- Bylund, D. B., Forte, L. R., Morgan, D. W. & Martinez, J. R. (1981) *J. Pharmacol. Exp. Ther.* **218**, 134–141
- Bylund, D. B., Martinez, J. R. & Pierce, D. L. (1982) *Mol. Pharmacol.* **21**, 27–35
- Creba, J. A., Downes, C. P., Hawkins, P. T., Brewster, G., Michell, R. H. & Kirk, C. J. (1983) *Biochem. J.* **212**, 733–747
- Downes, C. P. & Michell, R. H. (1981) *Biochem. J.* **198**, 133–140
- Downes, C. P. & Michell, R. H. (1982) *Cell Calcium* **3**, 467–502
- Ekstrom, J. (1980) *Acta Physiol. Scand.* **108**, 253–261
- Fleming, W. W. (1981) *Trends Pharmacol. Sci.* **2**, 152–154
- Hanley, M. R., Lee, C. M., Jones, L. M. & Michell, R. H. (1980) *Mol. Pharmacol.* **18**, 78–83
- Hokin, L. E. & Sherwin, A. K. (1957) *J. Physiol. (London)* **125**, 18–29
- Jolles, J., Wirtz, K. W. A., Schotman, P. & Gispén, W. H. (1979) *FEBS Lett.* **105**, 110–114
- Jones, L. M. & Michell, R. H. (1974) *Biochem. J.* **142**, 583–590
- Low, M. G. & Finean, J. B. (1977) *Biochem. J.* **167**, 281–284
- Michell, R. H. (1975) *Biochim. Biophys. Acta* **415**, 81–147
- Michell, R. H., Kirk, C. J., Jones, L. M., Downes, C. P. & Creba, J. A. (1981) *Philos. Trans. R. Soc. London Ser. B* **296**, 123–137
- Oron, Y., Lowe, M. & Selinger, Z. (1975) *Mol. Pharmacol.* **11**, 79–86
- Panagia, V., Michiel, D. F., Dhalla, K. S., Jijar, M. S. & Dhalla, N. S. (1981) *Biochem. Biophys. Acta* **676**, 395–400
- Pawlikowski, M. (1982) *Life Sci.* **30**, 313–320
- Putney, J. W. (1979) *Pharmacol. Rev.* **30**, 209–245
- Roelofsen, B. & Van Linde-Sibenius Trip, M. (1981) *Biochim. Biophys. Acta* **647**, 302–306
- Sherman, W. R., Leavitt, A. L., Honchar, M. P., Hallcher, L. M. & Phillips, B. E. (1981) *J. Neurochem.* **36**, 1947–1951
- Weiss, S. J. & Putney, J. W. (1981) *Biochem. J.* **194**, 463–468
- Weiss, S. J., McKinney, J. S. & Putney, J. W., Jr. (1982a) *Biochem. J.* **204**, 587–592
- Weiss, S. J., McKinney, J. S. & Putney, J. W., Jr. (1982b) *Biochem. J.* **206**, 555–560
- Wilborn, W. H. & Schneyer, C. A. (1972) *Z. Zellforsch.* **130**, 471–480
- Yandrasitz, J. R. & Segal, S. (1979) *FEBS Lett.* **108**, 279–282