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Similar Effects of Substance P and Related Peptides on Salivation and on Phosphatidylinositol Turnover in Rat Salivary Glands

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SUMMARY

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The effects of substance P and related peptides on salivation *in vivo* and on the incorporation of ^{3}H-inositol into rat salivary gland lipids *in vitro* were compared. Physalaemin was the most active peptide tested on both responses. Substance P was less potent, but was three- to sixfold more potent than peptides consisting of the C-terminal 6–10 residues of substance P. Both the C-terminal pentapeptide and the C-terminal deamidated substance P showed a dramatic reduction of potency in both assays. Substance P receptors exist in all three rat salivary glands, and a stimulation of ^{3}H-inositol incorporation was also observed in all three glands. The close correlation between the relative activities of the different substance P-related peptides in evoking salivation and ^{3}H-inositol incorporation strongly indicates that the same population of substance P receptors is involved in each of these two responses and is consistent with the idea that phosphatidylinositol breakdown may be a reaction involved in stimulus–response coupling at the substance P receptors of salivary glands. The incorporation of ^{3}H-inositol into parotid gland lipids could profitably be adopted as a simple and sensitive biochemical procedure for screening the biological activity of potential agonists and antagonists at substance P receptors.

INTRODUCTION

Substance P is a cationic undecapeptide (Arg–Pro–Lys–Pro–Gln–Gln–Phe–Phe–Gly–Leu–Met–NH_2) which appears likely to be a neurotransmitter both within the central nervous system and in neurones associated with the gastrointestinal tract. Although substance P receptors in the central nervous system appear to be excitatory (1), there is little detailed information on their mechanism of action (2).

Amongst the most potent actions of substance P in peripheral tissues is its stimulation of salivation (3). Closely associated with the salivary flow induced by secretagogues is a substantial efflux of K^+ from the salivary glands. Such an efflux is also produced by substance P *in vitro*, and this is brought about as a result of a substance P-induced elevation of the Ca^{2+} ion concentration in the cytosol compartment of the secretory cells. In the rat, responses of this type can be demonstrated in all three salivary glands, namely, the parotid, submaxillary, and sublingual (4–8).

In many tissues in which hormones and neurotransmitters bring about a rise in the cytosolic Ca^{2+} ion concentration, this is accompanied by an increase in the turnover of phosphatidylinositol. The initiating reaction for this biochemical response is phosphatidylinositol breakdown, and it has been suggested that this reaction might play an essential role in the coupling between receptor activation and the mobilisation of Ca^{2+} within cells (9, 10). Amongst the receptors which control this response are the substance P receptors of the rat parotid gland (11).

In the present study we have demonstrated that substance P can stimulate ^{3}H-inositol incorporation into the lipids of all three salivary glands, and we have used a variety of peptides related to substance P in order to compare the ligand selectivities of the substance P receptors that are responsible for salivation and for stimulation of phosphatidylinositol turnover. Our results suggest that the same receptors are probably responsible for both events and that stimulated labelling of phosphatidylinositol in rat parotid fragments can, therefore, be used as

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a simple and convenient biochemical assay to screen the biological activities of potential agonists and antagonists at substance P receptors.

MATERIALS AND METHODS

Materials. Synthetic substance P and its C-terminal decapeptide, nonapeptide, octapeptide, heptapeptide, hexapeptide, pentapeptide, tetrapeptide and tripeptide, C-terminal deamidated substance P, and physalaemin (Pyr-Ala-Asp-Pro-Asn-Lys-Phe-Tyr-Gly-Leu-Met-NH_2) were obtained from Peninsula Laboratories, San Carlos, Calif. When analysed by high-pressure liquid chromatography, all of these peptides except the heptapeptide and hexapeptide appeared homogeneous. The latter two peptide preparations were mixtures which appeared to include N-terminal pyroglutamyl forms and probably also molecules in which the C-terminal methionine was oxidized.

Collagenase was from Worthington Biochemicals.

Other materials were obtained from sources specified in earlier publications (12–14).

^{3}H-Inositol incorporation into tissue lipids. Parotid gland: The medium used throughout was a Krebs–Ringer bicarbonate buffer containing 10 mM inosine, 0.5 mM adenine, and 5 mM 3-hydroxybutyrate, gassed with 95% O_2:5% CO_2 (this medium is superior to a glucose medium for studies of the parotid gland; see Ref. 15). For a typical experiment involving 35–40 incubations, the parotid glands were removed from five male Sprague–Dawley rats, freed of contaminating lymph nodes and adipose and connective tissue, and kept in medium at room temperature. The glands were chopped three times at a spacing of 0.15 mm on a McIlwain tissue chopper (Mickle Instruments Ltd.), with the stage of the chopper being rotated through 60° between each treatment. The parotid fragments were then incubated for 30 min at 30°C in a solution of collagenase in Krebs–Ringer medium (0.5 mg/ml, 5 ml for each pair of parotid glands). This served to remove many of the collagen fibres which protrude from the tissue fragments and tend to link them together in the tissue suspensions. The collagenase-treated fragments were rinsed three times with medium and were allowed to settle out of suspension. After removal of most of the overlying medium, 50-μl samples of the concentrated suspension of parotid fragments were pipetted into flat-bottomed polypropylene vials (Beckman Biovals) containing 190 μl of medium to which had been added 1 μCi of 2-^{3}H-inositol (4.5 Ci/mmol; Radiochemical Centre, Amersham). The vials were gassed with 95% O_2:5% CO_2 and incubated at 37°C in a shaking water bath for 30 min, after which they were removed, additions of peptides were made in a total volume of 10 μl, and the vials were gassed again and returned to the bath for a further 30 min. Incubations were terminated by the addition of 3.75 vol (0.94 ml) of chloroform:methanol (1:2 by volume). After at least 15 min, 1.25 vol (0.31 ml) each of chloroform and of 2 M KCl containing 10 mM unlabelled inositol was added and the contents of the vials were thoroughly mixed. For determination of the incorporation of ^{3}H-inositol into lipids, 0.4 ml of the lower chloroform phase was removed, dried at 50°C, dissolved in a toluene-based PPO:POPOP scintillant, and counted in a Packard liquid scintillation counter. The background carry-over of ^{3}H-inositol into the chloroform phase during this procedure was normally about 500–800 cpm. Preliminary experiments established that all of the peptides being tested gave the same maximum stimulation of ^{3}H-incorporation as substance P, so a maximally stimulating concentration of substance P (between 0.6 and 3 μM) was included in every experiment, and the responses to other stimuli were calculated as a percentage of this maximum response.

Submaxillary and sublingual glands: The experimental design was identical to that used with parotid glands, except that the medium used was Krebs–Ringer bicarbonate containing 10 mM glucose. (Glucose and β-hydroxybutyrate media equal levels of ^{3}H-inositol incorporation with these two glands (unpublished results).) Each pair of sublingual glands was incubated in 2.5 ml of collagenase solution, and five rats provided sufficient sublingual tissue for about 15 incubations. The yield and incubation conditions for submaxillary fragments were similar to those for parotid fragments.

Measurement of salivation in vivo. Male Sprague–Dawley rats weighing 200–250 g were used. Animals were anaesthetized with Nembutal (60 mg/kg, i.p.). Tracheotomy was performed and the left jugular vein cannulated. Body temperature was monitored with a rectal thermometer and was maintained constant at 36°C with a table lamp. Peptides were dissolved in 0.9% NaCl containing 500 U heparin/ml, with the exception of the C-terminal hexapeptide and heptapeptide, which were first dissolved in 30% acetonitrile and then diluted to the required concentrations with 0.9% NaCl containing 500 U heparin/ml. Test materials were administered through the left jugular vein at 10- to 20-min intervals. Secretion of saliva was measured as the weight gained by absorbent cotton pellets placed in the animal's mouth every 2 min. The basal salivary secretion under these conditions was less than 2 mg/min.

Measurements of degradation of peptides. Parotid fragments were prepared and incubated with substance P exactly as for the assays of ^{3}H-inositol incorporation, except that the labelled inositol was omitted. At intervals, small samples of the medium bathing the fragments were withdrawn, enzyme activity was abolished by heating for 10 min at 100°C, and the remaining substance P was assayed by radioimmunoassay using an antiserum directed to the C-terminal sequence of the peptide (16).

RESULTS

Salivation. Of the peptides tested, physalaemin was the most active, being appreciably more potent than substance P (Table 1). The C-terminal decapeptide, octapeptide, heptapeptide, and hexapeptide all showed potencies between 0.16 and 0.4 times that of substance P. The C-terminal tripeptide and tetrapeptide were inactive at all doses tested, and the pentapeptide was at least 300 times less potent than substance P. All of the peptides which retained the C-terminal hexapeptide sequence were, with the exception of the nonapeptide, capable of eliciting a full salivation response with a dose–response

TABLE 1

Relative potencies of substance P and related peptides in evoking salivation and in stimulating ^{3}H-inositol incorporation into parotid gland lipids

Relative potencies (substance P = 1) were calculated from the concentrations of peptides which evoked half-maximal stimulation of salivation *in vivo* or of ^{3}H-inositol incorporation in parotid gland fragments. Substance P gave half-maximal salivation at approximately 2 μg/kg body weight and half-maximal ^{3}H-incorporation at approximately 17 nM.

	Relative potencies	
	Salivation	^{3}H-Inositol incorporation
Substance P	(1.0)	(1.0)
C-terminal deamidated substance P	0.0003	0.00016
Physalaemin	2.7	3.4
C-Terminal fragments of substance P		
Decapeptide (SP_{2-11})	0.26	0.26
Nonapeptide (SP_{3-11})	0.16[a]	0.33
Octapeptide (SP_{4-11})	0.37	0.28
Heptapeptide (SP_{5-11})	0.28	0.24
Hexapeptide (SP_{6-11})	0.17	0.38
Hexapeptide (N-terminal pyroglutamyl SP_{6-11})	0.40	Not tested
Pentapeptide (SP_{7-11})	0.003	0.00017
Tetrapeptide (SP_{8-11})	0.002	Not tested
Tripeptide (SP_{9-11})	0.002	Not tested

[a] This was the relative potency of the nonapeptide in evoking half of its maximum response, but this maximal response was less than that evoked by the other peptides (see text and Fig. 1).

curve parallel to the dose–response curve for substance P. The nonapeptide, however, behaved as a partial agonist and did not elicit a full response even at high concentrations (Fig. 1). Substance P which had been deamidated at its C-terminus did provoke a small salivation response, but it was not possible to inject sufficient amounts of this peptide to give a reliable estimate of its potency.

^{3}H-Inositol incorporation into parotid gland lipids. We previously showed that substance P stimulates phosphatidylinositol breakdown and the labelling of phosphatidylinositol with ^{32}P in rat parotid fragments (11). In addition, we found that physalaemin produced a similar

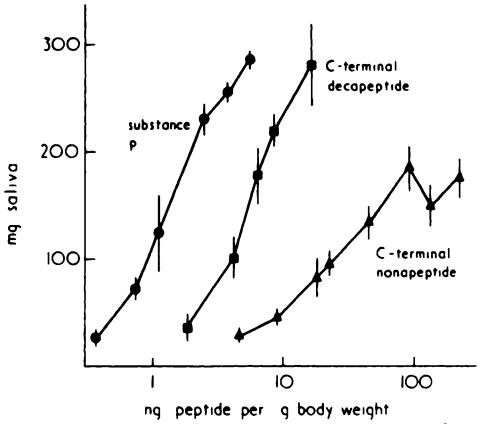

FIG. 1. *The dose–response curves for salivation elicited by substance P and its C-terminal decapeptide and nonapeptide*

Results are presented as means ± SEM of three to eight values.

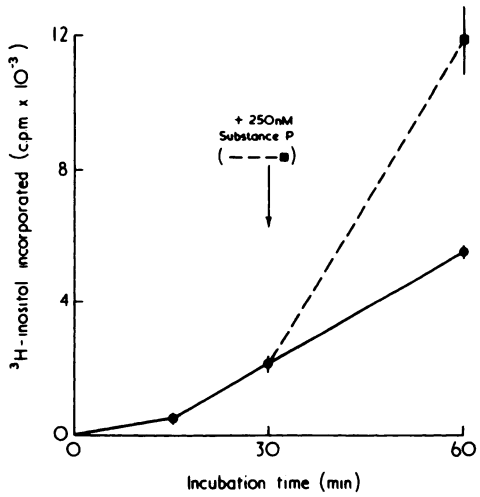

FIG. 2. *Stimulation of ^{3}H-inositol incorporation into the lipids of parotid gland fragments*

Fragments were incubated for 60 min with ^{3}H-inositol, with 250 nM substance P present in some incubations from 30 to 60 min. Results are from a typical experiment and are means ± SEM of four replicate incubations.

response, assessed by the incorporation of ^{3}H-inositol into phosphatidylinositol (17).

When salivary gland fragments are incubated for brief periods with ^{3}H-inositol, the only lipid which shows significant incorporation of the labelled precursor is phosphatidylinositol, and this led us to develop the simplified assay for stimulated phosphatidylinositol metabolism that we have utilised in the present studies. Figure 2 shows a set of results typical of those obtained with this protocol. There is an appreciable lag period during which little incorporation occurs, probably reflecting the time taken for the labelled precursor to equilibrate with the intracellular inositol pool, after which incorporation in the unstimulated glands is approximately linear with time. Stimulation by a supramaximal concentration of substance P then initiates a substantial increase in the incorporation of ^{3}H-inositol into lipids: In a large number of experiments the total ^{3}H-inositol incorporation in the substance P-stimulated fragments was from 131 to 230% of the incorporation in unstimulated fragments (Table 2).

Phosphatidylinositol turnover in parotid glands can also be stimulated effectively by either muscarinic or α-adrenergic stimulation (12, 13, 18). However, in the present study preincubation of parotid fragments for 15 min with either 10 μM atropine or 10 μM phenoxybenzamine caused no change in ^{3}H-inositol incorporation either in unstimulated fragments or in the presence of substance P (results not shown). In addition, we tested a variety of other pharmacologically active peptides, none of which produced any change in the incorporation of ^{3}H-inositol into parotid lipids even at concentrations of ≥1 μM: These were somatostatin, neurotensin, bradykinin, pancreozymin, bombesin, angiotensin II, and VIP (vasoactive intestinal peptide).

In our previous study, we found that maximum stimulation of phosphatidylinositol turnover by substance P occurred at about 50–100 nm (11, 17). This was confirmed

TABLE 2

Substance P-stimulated ^{3}H-inositol incorporation into salivary gland lipids

Tissue fragments were incubated for 60 min with ^{3}H-inositol. When added, substance P was present at a concentration of between 0.6 and 3 μM for the final 30 min. Incubations were in triplicate or quadruplicate, and the results presented, which represent the inositol incorporation in stimulated tissue as a percentage of the incorporation in appropriate control incubations, are means ± SEM for the number of experiments denoted in parentheses.

	^{3}H-Inositol incorporation in the presence of substance P
	% of control
Parotid gland	172 ± 5 (22)
Submaxillary gland	120 ± 5 (5)
Sublingual gland	121 ± 2 (4)

in the present study (Fig. 3), indicating that collagenase treatment of the tissue fragments did not substantially alter the sensitivity of the parotid gland to substance P. Half-maximal stimulation of phosphatidylinositol labelling occurred at about 17 nM substance P. As with salivation *in vivo*, physalaemin was appreciably more active than substance P, and peptides which retained the C-terminal hexapeptide sequence of substance P showed potencies about one-third to one-fifth that of substance P (Fig. 3 and Table 1). This parallel with the *in vivo* salivation results also held for the C-terminal pentapeptide and for C-terminal deamidated substance P, which were both very low in potency. With ^{3}H-inositol incorporation, however, concentrations were achieved at which these peptides produced the maximal effect of substance P. There was one substantial difference between the relative behaviours of the peptides in the two assays: The C-terminal nonapeptide evoked a maximum increase in ^{3}H-inositol incorporation similar to that caused by substance P, whereas even at high doses it elicited less salivation than the other peptides *in vivo* (Figs. 1 and 3).

^{3}H-Inositol incorporation into submaxillary and sublingual gland lipids. When incubated and stimulated under essentially the same conditions as the parotid fragments, collagenase-dispersed fragments of these two glands also showed increased incorporation of ^{3}H-inositol into lipids when stimulated with substance P (Table 2). However, the increases in labelling in these two glands were substantially smaller than in the parotid, thus precluding the same detailed structure–activity studies. The following important points were, however, established. First, in neither gland was the substance P response modified by 10 μM atropine, again suggesting a direct effect of substance P on the acinar cells rather than some indirect effect mediated through their cholinergic innervation. Second, limited studies in the submaxillary gland with substance P, and C-terminal decapeptide, and the C-terminal nonapeptide revealed behaviour similar to that seen in the parotid: All three peptides elicited the same full response, and substance P was more potent than the truncated peptides (results not shown).

Degradation of substance P. Only limited studies were undertaken, since some unidentified material present in the parotid fragments appeared to exhibit a substance P-binding activity which interfered with the radioimmunoassay technique. However, the limited information which was gathered clearly indicated that when substance P was added to parotid fragments to a final concentration of 100 nM, then most was degraded within the first 5–10 min. In addition, parallel incubations in which 100 nM substance P and 100 nM C-terminal decapeptide were compared showed no obvious differences in their rates of degradation.

DISCUSSION

In general, the substance P receptors in rat salivary

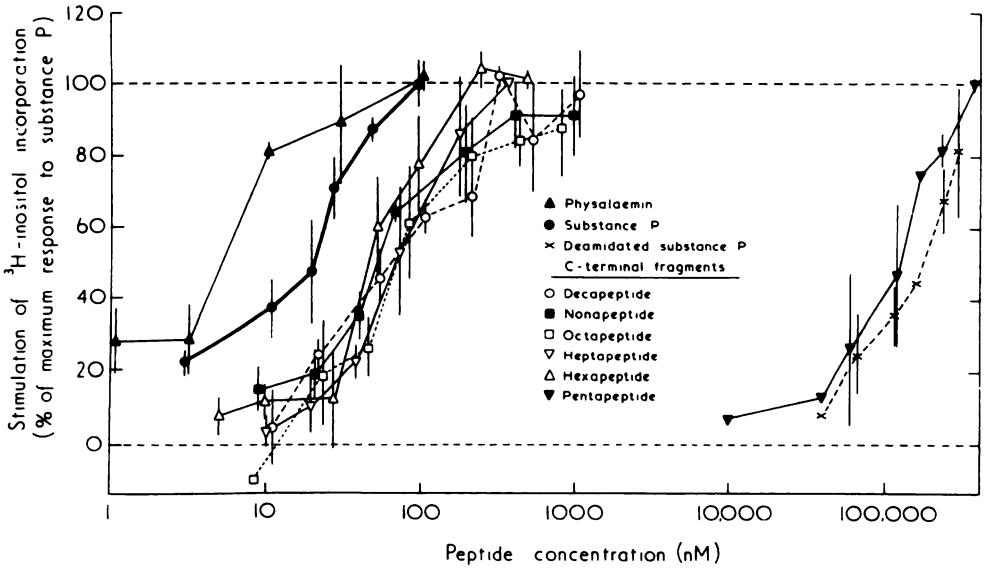

FIG. 3. *Stimulation of ^{3}H-inositol incorporation into the lipids of parotid gland fragments by substance P and related peptides*

In each experiment, the responses to each peptide were measured in triplicate or quadruplicate, and the mean values are expressed as percentages of the maximum response evoked by 0.6–3 μM substance P. Each point represents the mean ± SEM of the average values (each from three or four replicates) in one to seven separate experiments (most points are derived from three to five experiments).

glands, whether responding *in vivo*, and giving salivation, or *in vitro*, and stimulating the incorporation of ^{3}H-inositol into lipids, appear to recognise the same key features of the substance P molecule as do the substance P receptors in guinea pig ileum or in spinal neurones (see Ref. 2). The attributes which confer high potency upon a ligand are the retention of the C-terminal hexapeptide sequence of native substance P, with a permissible substitution of certain hydrophobic amino acid residues at position 4, and the amidation of the C-terminal methionine residue (2). Loss of either of these features of the molecule leads to a precipitous fall in agonist potency. However, the rat salivary gland responses show one characteristic that is not observed in the other systems, namely, a reduction in agonist potency on removal of the N-terminal arginine residue from substance P. Since the rates of degradation of substance P and the decapeptide did not appear to be markedly different, this distinction seems likely to be due to a real difference in their interaction with the substance P receptors.

In considering the functional relationships between the salivation response and the labelling of lipids with ^{3}H-inositol, an obvious concern might be that the results of our experiments on substance P degradation indicated that active peptides were present only at the added concentrations during the early part of the stimulated ^{3}H-inositol incorporation. On the basis of the transience of substance P-stimulated K^+ efflux, Friedman and Selinger (7) have also concluded that fragments of parotid rapidly degrade added substance P. However, it is now well established that the initiating event in receptor-stimulated phosphatidylinositol metabolism is phosphatidylinositol breakdown, with the enhanced synthesis of phosphatidylinositol following as a compensatory event which presumably serves to replenish the phosphatidylinositol pools of the cell. This is true both for substance P (11) and for many other stimuli (14). The stimulation of ^{3}H-inositol incorporation seen in the present experiments is therefore likely to be a reasonably accurate reflection of the amount of phosphatidylinositol breakdown that occurred during the first few minutes of stimulation. In accord with this view, we found that neither the presence of excess ligand at concentrations that would sustain full stimulation for a substantial period nor repeated additions of stimuli during the incubation could enhance the stimulated rates of ^{3}H-inositol incorporation.

With the exception of the anomalous behaviour of the C-terminal nonapeptide, the correspondence between the structure–activity relationships for salivation and for ^{3}H-inositol incorporation is remarkably close. One is, therefore, led to the conclusion that the same population of substance P receptors is probably responsible for controlling both of these responses. Also consistent with this view is the observation that all three of the rat's salivary glands, namely, parotid, submaxillary, and sublingual, showed increased phosphatidylinositol labelling when stimulated with substance P, a result predicted by the fact that each isolated gland possesses substance P receptors capable of evoking a Ca^{2+}-dependent K^+ efflux (4–8). Although these data do not establish with any certainty the role of substance P-stimulated phosphatidylinositol breakdown, they are consistent with the suggestion that is might be an essential reaction in stimulus–response coupling at this receptor (11).

One of the more interesting general possibilities that arises from the present study is that stimulation of phosphatidylinositol metabolism may prove of general applicability as a convenient biochemical measure with which to assess the biological activities of potential ligands at any Ca^{2+}-mobilising receptor. If phosphatidylinositol breakdown is, as has been suggested (9–11, 21, 22), an event that is essential to stimulus–response coupling at such receptors and is, therefore, closely coupled to receptor activation, then its use as a measure of receptor activation offers obvious advantages over the measurement of more distal events such as muscle contraction, K^+ efflux, or salivation. Moreover, it is, with the simplified methodology employed here, a response whose assay is extremely rapid and convenient.

As with any simple screening assay, this method should be applied only with the knowledge that there are situations in which apparent false positive or false negative results might be obtained. For example, increased phosphatidylinositol labelling can be produced by amphiphilic cationic drugs that stimulate the synthesis de novo of this lipid (19) or in situations that lead to diacylglycerol liberation from lipids other than phosphatidylinositol, e.g., an increase in the intracellular Ca^{2+} ion concentration leading to polyphosphoinositide breakdown (10) or possibly after treatment of cells externally with phospholipases (e.g., Ref. 20). Similarly, a reduction in the receptor-stimulated incorporation of ^{3}H-inositol might be caused either by receptor-mediated antagonism or by other mechanisms that would interfere more directly with cellular lipid metabolism. Any unexpected effect of a putative receptor ligand should, therefore, be rigorously checked by a more detailed analysis of the changes in lipid metabolism that might underlie apparently simple changes in ^{3}H-inositol incorporation and, whenever possible, by comparison with other receptor-specific events (e.g., radioligand binding).

With respect to its particular use with the substance P receptor of the parotid, the specific advantages of the ^{3}H-inositol incorporation assay include the following: the removal of potential complexities arising from any whole animal assay (e.g., the anomalous behavior of the C-terminal nonapeptide of substance P *in vivo*), the ability to assess quantitatively the activities of both agonists and potential antagonists, the accessibility of realistic measurements of biological activity even for ligands with receptor affinities too low for them to be assessed conveniently *in vivo*, and finally, the achievement of all of these measurements using only very small total amounts of ligands.

REFERENCES

1. Otsuka, M., and S. Konishi. Substance P an excitatory transmitter of primary sensory neurons. *Cold Spring Harbor Symp. Quant. Biol.* **40**: 135–143 (1976).
2. Hanley, M. R., and L. Iversen. Substance P receptors, in *Neurotransmitter Receptors* (H. Yamamura and S. Enna, eds.). Chapman & Hall, London (1980) (in press).
3. Chang, M. M., and S. E. Leeman. Isolation of a sialogogic peptide from bovine

hypothalamic tissue and its characterisation as Substance P. *J. Biol. Chem.* **245:** 4784–4790 (1970).
4. Spearman, T. N., and E. T. Pritchard. Potassium release from submandibular salivary gland in vitro. *Biochim. Biophys. Acta* **466:** 198–207 (1977).
5. Putney, J. W. Muscarinic, alpha-adrenergic and peptide receptors regulate the same calcium influx sites in the parotid gland. *J. Physiol.* **268:** 139–149 (1977).
6. Putney, J. W., B. A. Leslie and S. H. Marier. Calcium and the control of potassium efflux in the sublingual gland. *Am. J. Physiol.* **235:** C128–C135 (1978).
7. Friedman, Z. Y., and Z. Selinger. A transient release of potassium mediated by the action of substance P on rat parotid slices. *J. Physiol.* **278:** 461–469 (1978).
8. Rudich, L., and F. R. Butcher. Effect of substance P and eledoisin on K^+ efflux, amylase release and cyclic nucleotide levels in slices of rat parotid gland. *Biochim. Biophys. Acta* **444:** 704–711 (1976).
9. Michell, R. H. Inositol phospholipids and cell surface receptor function. *Biochim. Biophys. Acta* **415:** 81–147 (1975).
10. Michell, R. H. Inositol lipids in membrane function. *Trends Biochem. Sci.* **4:** 128–131 (1979).
11. Jones, L. M., and R. H. Michell. Enhanced phosphatidylinositol breakdown as a calcium-independent response of rat parotid fragments to substance P. *Biochem. Soc. Trans.* **6:** 1035–1037 (1978).
12. Michell, R. H., and L. M. Jones. Enhanced phosphatidylinositol labelling in rat parotid fragments exposed to α-adrenergic stimulation. *Biochem. J.* **138:** 47–52 (1974).
13. Jones, L. M., and R. H. Michell. Breakdown of phosphatidylinositol provoked by muscarinic cholinergic stimulation of rat parotid-gland fragments. *Biochem. J.* **142:** 583–590 (1974).
14. Jones, L. M., S. Cockcroft and R. H. Michell. Stimulation of phosphatidylinositol turnover in various tissues by cholinergic and adrenergic agonists, by histamine and by caerulein. *Biochem. J.* **182:** 669–676 (1979).
15. Batzri, S., and Z. Selinger. Enzyme secretions mediated by the epinephrine β-receptor in rat parotid slices. *J. Biol. Chem.* **248:** 356–360 (1973).
16. Powell, D., S. E. Leeman, G. W. Tregear, H. D. Niall and J. T. Potts. Radioimmunoassay for substance P. *Nature New Biol.* **241:** 252–254 (1973).
17. Jones, L. M. *The Mechanism and Function of Stimulated Phosphatidylinositol Metabolism in Rat Parotid Gland and Other Tissues*, Ph.D. thesis. University of Birmingham (1978).
18. Oron, Y., M. Lowe and Z. Selinger. Incorporation of inorganic [^{32}P]phosphate into rat parotid phosphatidylinositol: Induction through activation of alpha adrenergic and cholinergic receptors and relation to K^+ release. *Mol. Pharmacol.* **11:** 79–86 (1975).
19. Allan, D., and R. H. Michell. Enhanced synthesis de novo of phosphatidylinositol in lymphocytes treated with cationic amphiphilic drugs. *Biochem. J.* **148:** 471–478 (1975).
20. Allan, D., M. G. Low, J. B. Finean and R. H. Michell. Changes in lipid metabolism and cell morphology following attack by phospholipase C on erythrocytes or lymphocytes. *Biochim. Biophys. Acta* **413:** 309–316 (1975).
21. Michell, R. H., S. S. Jafferji and L. M. Jones. Receptor occupancy doseresponse curve suggests that phosphatidylinositol breakdown may be intrinsic to the mechanism of the muscarinic cholinergic receptor. *FEBS Lett.* **69:** 1–5 (1976).
22. Jones, L. M., and R. H. Michell. Stimulus-response coupling at α-adrenergic receptors. *Biochem. Soc. Trans.* **6:** 673–688 (1978).

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