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Rapid accumulation of inositol phosphates in isolated rat superior cervical sympathetic ganglia exposed to V_1-vasopressin and muscarinic cholinergic stimuli

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1. An accumulation of ^{3}H-labelled inositol phosphates is observed when prelabelled rat superior cervical sympathetic ganglia are exposed to [8-arginine]vasopressin or to muscarinic cholinergic stimuli. The response to vasopressin is much greater than the response to cholinergic stimuli. 2. The response to vasopressin is blocked by a V_1-vasopressin antagonist, and oxytocin is a much less potent agonist than vasopressin. Vasopressin causes no increase in the cyclic AMP content of ganglia. These ganglia therefore appear to have functional V_1-vasopressin receptors that are capable of activating inositol lipid breakdown, but no V_2-receptors coupled to adenylate cyclase. 3. The first [^{3}H]inositol-labelled products to accumulate in stimulated ganglia are inositol trisphosphate and inositol bisphosphate, suggesting that the initiating reaction in stimulated inositol lipid metabolism is a phosphodiesterase-catalysed hydrolysis of phosphatidylinositol 4,5-bisphosphate (and possibly also phosphatidylinositol 4-phosphate). 4. This response to exogenous vasopressin occurs in ganglia incubated in media of reduced Ca^{2+} concentration. 5. The physiological functions of the V_1-vasopressin receptors of these ganglia remain unknown.

Activation of a wide variety of cell surface receptors (e.g. muscarinic cholinergic, H_1-histamine, α_1-adrenergic, V_1-vasopressin) causes a stimulation of inositol lipid metabolism in many tissues. In general, the effective receptors are those that bring about a rise in cytosol Ca^{2+} concentration, and perhaps also activate protein kinase C, in stimulated cells (Michell, 1975, 1979, 1983; Berridge, 1980, 1981; Putney, 1981; Michell & Kirk, 1981*a*; Michell *et al.*, 1981; Nishizuka, 1983; Rink *et al.*, 1983). For many years it was thought that the initiating reaction in stimulated inositol lipid metabolism was a phosphodiesterase-catalysed breakdown of PtdIns, but recent studies suggest that the major, and possibly the only, initiating event is phosphodiesterase-catalysed hydrolysis of PtdIns4,5P_2) (Akhtar & Abdel-Latif, 1980; Michell *et al.*, 1981, 1984; Kirk *et al.*, 1981*a*; Weiss *et al.*, 1982; Berridge, 1983, 1984; Creba *et al.*, 1983; Berridge *et al.*, 1983; Putney *et al.*, 1983; Thomas *et al.*, 1983; Rebecchi & Gershengorn, 1983; Downes & Wusteman, 1983).

Although the first studies of receptor-stimulated metabolism of inositol phospholipids were primarily devoted to studies of exocrine pancreas (Hokin & Hokin, 1953, 1954), they also included brief mention of a similar effect of acetylcholine upon inositol lipid metabolism in slices of cerebral cortex. Since that time, there have been many reports of stimulated inositol lipid metabolism in various types of nervous tissues, including sympathetic ganglia (for reviews, see Hokin, 1969; Michell, 1975, 1981, 1982; Hawthorne & Pickard, 1979; Downes, 1982, 1983). The earliest studies of sympathetic ganglia employed cholinergic agonists and either a natural or imposed stimulation of the afferent nerve (Hokin *et al.*, 1960; Larrabee *et al.*, 1963; Larrabee & Leicht, 1965; Larrabee, 1968), each of which stimulated inositol lipid metabolism through activation of muscarinic cholinergic receptors (Lapetina *et al.*, 1976; Pickard *et al.*, 1977).

Abbreviations used: PtdIns, phosphatidylinositol; Ins*P*, InsP_2, InsP_3, the mono-, bis- and tris-phosphates of inositol; PtdIns4*P*, phosphatidylinositol 4-phosphate; PtdIns4,5P_2, phosphatidylinositol 4,5-bisphosphate; Gro*P*Ins, glycerophosphoinositol; [Arg^8]vasopressin, [8-arginine]vasopressin; VLP, vasopressin-like peptide.

However, it was also shown many years ago that depolarization with high extracellular K^+ can stimulate inositol lipid metabolism in ganglia, even after axotomy (Nagata *et al.*, 1973). The most obvious interpretation of this result is that depolarization causes release within the ganglia of a non-cholinergic neurotransmitter that is capable of stimulating inositol phospholipid breakdown. In addition, Lakshmanan (1978) has shown a stimulation of inositol lipid metabolism in neonatal ganglia by nerve growth factor.

Recent studies have demonstrated the presence of a number of putative peptide neurotransmitters in sympathetic ganglia from various species: these include gonadotropin-releasing hormone (GnRH, LHRH, Jan *et al.*, 1980; Kuffler, 1980), substance P (Konishi *et al.*, 1980), enkephalins (Schultzberg *et al.*, 1979) and neuropeptide Y (Lundberg *et al.*, 1982). We therefore decided to screen a variety of peptides, including those that stimulate inositol lipid metabolism in peripheral tissues, in order to determine whether they would affect inositol lipid metabolism in isolated rat superior cervical sympathetic ganglia. The results reported here show that vasopressin provokes a very rapid degradation of inositol phospholipids in this tissue, almost certainly initiated by phosphodiesterase-catalysed breakdown of PtdIns4,5P_2. This unexpected observation provoked a search for, and the successful identification of, a vasopressin-like material both in these ganglia and in the nerve fibres that innervate sympathetically controlled tissues (Hanley *et al.*, 1984).

Materials and methods

Labelling of ganglia with [^3H]inositol, extraction of water-soluble inositol phosphates and their separation by anion-exchange chromatography

Superior cervical ganglia were dissected from male Wistar rats (200–250g) anaesthetized with ether. The afferent and efferent nerves were cut off and the connective tissue sheaths removed. Each ganglion was incubated for 2.5h at 37°C in a shaking incubator, in 0.3ml of Krebs–Ringer bicarbonate medium at pH7.2, containing 0.9mM-Ca^{2+} (unless otherwise specified), 10mM-glucose and 7.5μCi of *myo*-[2-^{3}H]inositol (16.9Ci/mmol; The Radiochemical Centre, Amersham, Bucks., U.K.). Incubations were gassed with O_2/CO_2 (19:1) every 30min. Ganglia were then transferred to a similar medium containing no inositol and the incubations continued. After 1h, 10mM-LiCl was added so as to inhibit the hydrolysis of any inositol phosphates formed during stimulation (Berridge *et al.*, 1982), and 5min later the appropriate agonist was added and incubation continued for the period specified. When receptor antagonists were to be tested they were added to incubations at the same time as LiCl, i.e. 5min before the agonists.

Incubations were terminated by the addition of 0.1ml of 10% (w/v) $HClO_4$. The acid extract was neutralized with 1.53M-KOH, and buffered to pH7.2 with 75mM-Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid]. $KClO_4$ was precipitated at 0°C for 90min on ice and removed by brief centrifugation. Samples (0.45ml) of the neutralized extracts were diluted to 5ml with 5mM-sodium tetraborate/0.5mM-EDTA and applied to columns (4cm × 0.6cm) of Dowex-1 X10 (100–200 mesh, formate form; Fluka AG, Buchs, Switzerland). Free inositol was eluted with 20ml of water, Gro*P*Ins with 15ml of 5mM-sodium tetraborate/60mM-ammonium formate, Ins*P* with 20ml of 5mM-sodium tetraborate/150mM-ammonium formate, InsP_2 with 20ml of 0.1M-formic acid/0.4M-ammonium formate, and InsP_3 with 20ml of 0.1M-formic acid/1M-ammonium formate. In order to determine the radioactivity of each fraction by liquid-scintillation counting, 2ml samples of the column eluates were mixed with 15ml of a xylene/Triton X-100 (2:1, v/v) scintillation fluid containing 2,5-diphenyloxazole (4g/l) and 1,4-bis(5-phenyloxazol-2-yl)benzene (0.1g/l). This procedure for extraction of water-soluble components, removal of $HClO_4$ and separation of inositol phosphates was validated by the use of tissue extracts to which ^{32}P-labelled samples of the individual inositol phosphates had been added (S. Palmer & P. T. Hawkins, unpublished work).

Extraction of lipids

Lipids were extracted from the acid-treated ganglia with chloroform/methanol/12M-HCl (100:200:1, by vol.). The washed lipid extracts were evaporated to dryness in counting vials and their total radioactivities determined. In the majority of experiments reported, the amounts of labelled inositol phosphates produced are expressed in terms of a standard incorporation of 10^5d.p.m. into the lipids of one ganglion.

Measurements of ganglionic cyclic AMP levels

Ganglia were incubated in 0.4ml of Krebs–Ringer bicarbonate medium for 30min at 37°C and were then transferred to 0.45ml of the same medium containing 10mM-theophylline as an inhibitor of cyclic nucleotide phosphodiesterases. Agents to be tested were added in 50μl of incubation buffer after 10min and incubations were continued for a further 15min. Each ganglion was then homogenized in 1ml of ice-cold 6% (w/v) trichloroacetic acid. The protein precipitate was removed by centrifugation. Trichloroacetic acid was extracted with 6 × 1ml of diethyl ether, and the

resulting aqueous sample was freeze-dried. The dried residues were reconstituted with either 110 μl (adrenaline-stimulated ganglia) or 55 μl (control and vasopressin-stimulated ganglia) of assay buffer containing 50mM-Tris/HCl (pH 7.4 at 20°C), 8mM-theophylline, 6mM-mercaptoethanol and 6mM-tetrasodium EDTA. Cyclic AMP was assayed in 50 μl samples of these extracts (in duplicate on the adrenaline-treated samples) by using the binding protein assay of Brown *et al.* (1971). The binding protein was a gift from Dr. G. Smith, Department of Medicine, Hope Hospital, Salford, U.K. [8-^{3}H]Cyclic AMP (20–30Ci/mol) was from The Radiochemical Centre, Amersham.

Other materials

[1-(β-Mercapto-β,β-cyclopentamethylenepropionic acid), 8-arginine]vasopressin was a gift from Professor M. Manning, Medical College of Ohio, Toledo, OH, U.S.A. [Arg^8]vasopressin, oxytocin, adrenaline, carbamyl-β-methylcholine (bethanechol), atropine and cyclic AMP were from Sigma Chemical Co.

Results

Previous studies of stimulated inositol lipid metabolism in sympathetic ganglia have always employed measurements of agonist-induced changes in the labelling of PtdIns in stimulated ganglia, despite the fact that the initiating reaction in such responses is almost certainly the breakdown of PtdIns4,5P_2 (see the Introduction). Stimulation of PtdIns labelling is a poor assay for such responses, both because it is only an indirect reflection of the occurrence of PtdIns4,5P_2 breakdown and because stimulation of the labelling of PtdIns can also be produced by a variety of other mechanisms (Michell, 1975; Michell & Kirk, 1981*b*). Assay of the labelled inositol phosphates liberated during lipid breakdown is both more sensitive and more specific than other available techniques for measuring breakdown of inositol lipids (Berridge *et al.*, 1982, 1983; Berridge, 1983). In particular, the use of Li^+ to inhibit inositol 1-phosphate phosphatase (Hallcher & Sherman, 1980; Berridge *et al.*, 1982) allows a large proportion of the liberated inositol lipid headgroups to be trapped as Ins*P* during sustained incubations. We have employed this approach in the experiments reported here, and in addition we have undertaken incubations for short periods so as to examine the inositol phosphates that are first released within the ganglia upon stimulation.

Muscarinic cholinergic stimulation of the accumulation of labelled inositol phosphates

Incubation of rat superior cervical ganglia for 60min with a high concentration (100 μM) of bethanechol, an agonist with predominantly muscarinic actions, caused an appreciable accumulation of inositol phosphates in the tissue. As expected after prolonged stimulation in the presence of Li^+, most of the accumulated radioactivity was found as Ins*P*, but there was also a significant increase in the amount of labelled InsP_3 (Table 1). This appears to provide confirmation that the initiating event in cholinergic stimulation of inositol lipid metabolism in these ganglia is the hydrolysis of an inositol lipid, and the presence of InsP_3 amongst these products points strongly to an attack upon PtdIns4,5P_2.

Incubation of ganglia with atropine, a muscarinic antagonist, had no effect upon the amounts of inositol phosphates accumulated during 60min of incubation in the absence of any agonist. This sug-

Table 1. *Accumulation of inositol phosphates in ganglia exposed to muscarinic cholinergic stimulation or to vasopressin*
Ganglia were labelled with [2-^{3}H]inositol. After preincubation for 5min with 10mM-LiCl in the presence or absence of 10 μM-atropine, ganglia were stimulated with 100 μM-bethanechol or 0.32 μM-[Arg^8]vasopressin for 60min. The values given are means ± S.E.M., corrected to a standard incorporation of 10^5 d.p.m. into the lipids of each ganglion. Significantly different from control incubations: * $P<0.05$, ** $P<0.001$; significantly different from incubations with atropine: ‡ $P\sim0.05$, ‡‡ $P<0.001$.

Conditions	^{3}H(d.p.m./10^5 d.p.m.) in: GroPIns	Ins*P*	InsP_2	InsP_3
Control ($n=7$)	3150 ± 250	21500 ± 2800	13800 ± 2900	990 ± 100
100 μM-Bethanechol ($n=9$)	3610 ± 290	40600 ± 3700**	18800 ± 2300	1350 ± 110*
10 μM-Atropine ($n=5$)	3530 ± 570	25800 ± 3400	12300 ± 3200	1220 ± 240
100 μM-Bethanechol and 10 μM-atropine ($n=7$)	3020 ± 210	11500 ± 1700‡‡	7400 ± 1500	740 ± 110‡
0.32 μM-[Arg^8]Vasopressin ($n=8$)	2470 ± 230	130300 ± 12800**	47200 ± 5200**	6830 ± 780**

gests that little muscarinic activation occurs in these incubated ganglia as a result of endogenous release of acetylcholine (Table 1). Atropine did, however, abolish the accumulation of inositol phosphates caused by bethanechol, thus confirming that this is a response mediated by muscarinic receptors. Indeed, the amounts of inositol phosphates accumulated in the presence of both bethanechol and atropine were lower than the amounts accumulated in the presence of atropine alone. We do not know the reason for this, but it is possible that in the presence of atropine the minor nicotinic activity of bethanechol predominates over its normally dominant muscarinic action and that nicotinic stimulation inhibits the breakdown of inositol lipids.

Effects of [Arg^8]vasopressin and oxytocin on the accumulation of labelled inositol phosphates

Incubation of ganglia with [Arg^8]vasopressin produced a much larger increase in inositol phosphate levels than did muscarinic cholinergic stimulation (Table 1). Fig. 1 shows that this increase was initiated within minutes and was sustained for at least 60min. Analysis of the individual phosphates showed that the levels of InsP_2 and InsP_3 were significantly elevated within 15s, but that no significant accumulation of Ins$P$ occurred until after the first minute (Table 2).

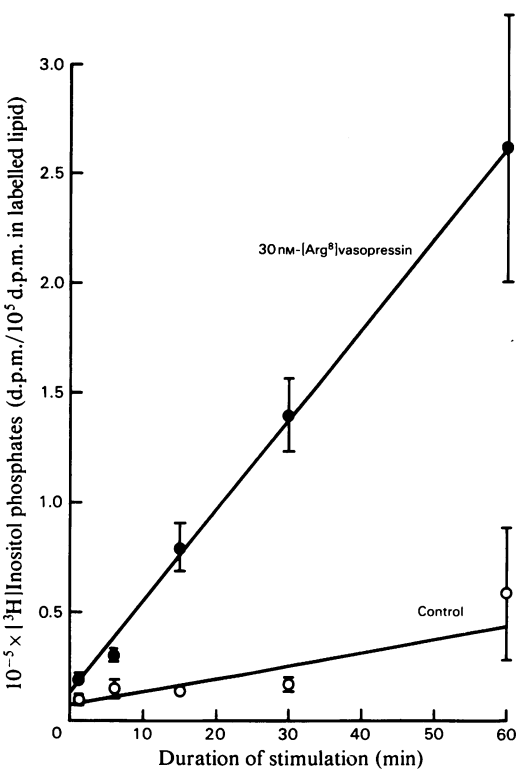

Fig. 1. *Time course of inositol phosphate accumulation in sympathetic ganglia incubated with 0.032 μM-[Arg^8]vasopressin*

Incubations, conditions and analyses were as in Table 2, except that all of the inositol phosphates were collected as a single fraction and their radioactivity determined.

The concentration of [Arg^8]vasopressin that was needed to provoke half-maximal accumulation of InsP_3, one of the most immediate consequences of receptor stimulation, was approx. 7nM (Fig. 2*a*). This is very similar to the concentration of this peptide that causes half-maximal depletion of PtdIns (Kirk *et al.*, 1981*b*) and of PtdIns4,5P_2 (Creba *et al.*, 1983; Thomas *et al.*, 1983) in isolated rat hepatocytes. The dose–response curve for InsP_2 was essentially identical to that for InsP_3 production (Fig. 2*b*), but that for Ins$P$ production appeared to be flatter and slightly displaced to lower vasopressin concentrations (Fig. 2*c*).

Stimulation of inositol lipid metabolism by vasopressin in other tissues has always been found to be a response to activation of Ca^{2+}-mobilizing V_1-receptors rather than to the V_2-receptors that activate adenylate cyclase (Kirk *et al.*, 1979, 1981*b*; Takhar & Kirk, 1981; Michell *et al.*, 1979; Creba *et al.*, 1983). Responses mediated by V_1-receptors are susceptible to inhibition by specific V_1 antagonists such as [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid), 8-arginine]vasopressin. This compound abolished the accumulation of inositol phosphates in vasopressin-stimulated ganglia (Table 3).

Although the two related peptides vasopressin and oxytocin are both capable of triggering Ca^{2+}-mediated responses (e.g. contraction) in their target tissues, they seem to do so through separate receptors: V_1-vasopressin receptors generally require about 100-fold more oxytocin than [Arg^8]-vasopressin in order to display a similar response. This was also true of the accumulation of the three inositol phosphates in ganglia (Figs. 2*a*, 2*b* and 2*c*). The maximum amount of InsP_3 accumulated in response to oxytocin appeared to be appreciably lower than with [Arg^8]vasopressin, as was previously observed with stimulated PtdIns labelling in liver cells (Kirk *et al.*, 1979), but this distinction between the two peptides was not apparent with the other inositol phosphates.

Stimulation of the accumulation of inositol phosphates in ganglia might, in principle, be a response either to primary activation of responsive cells by vasopressin or to a neurotransmitter released from these stimulated cells. This was investigated by stimulating ganglia incubated in a Ca^{2+}-free medium (with or without 1 μM-nifedipine, an antagonist of potential-sensitive Ca^{2+}

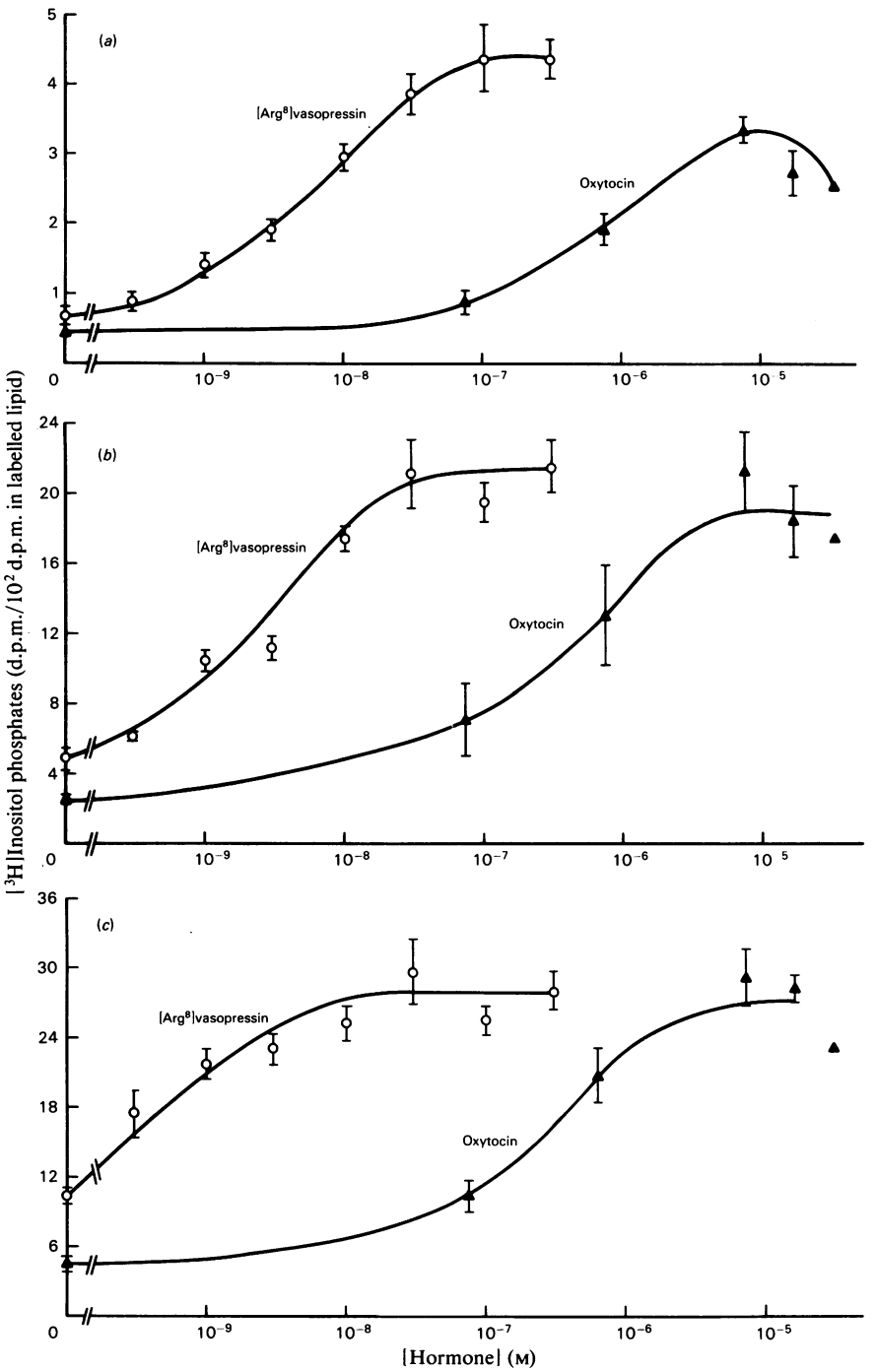

Fig. 2. *Concentration-dependence of [Arg⁸]vasopressin- and oxytocin-stimulated accumulation of inositol phosphates in rat superior cervical ganglia*

Isolated ganglia were labelled with [³H]inositol as described in the text. Following incubation in Krebs–Ringer medium containing 10mM-LiCl for 5min the ganglia were stimulated with [Arg⁸]vasopressin or oxytocin for 10min. Incubations were terminated and inositol phosphates separated as described in the text. The radioactivity of each phosphate ester was corrected to a standard incorporation of 10^2 d.p.m. into ganglionic lipids. The results presented are means ± S.E.M. ($n = 4$–8). (*a*) InsP_3, (*b*) InsP_2, (*c*) Ins$P$.

Table 2. *Time-course of the accumulation of inositol phosphates in [Arg^8]vasopressin-stimulated sympathetic ganglia*

Isolated ganglia prelabelled with [2-^{3}H]inositol were incubated in the presence and absence of 0.32 μM-[Arg^8]vasopressin for the appropriate time. Incubations were terminated and the inositol phosphates separated as described in the text. The values are means ± S.E.M. (numbers of ganglia analysed in parentheses) and are not corrected for incorporation of [^{3}H]inositol into ganglionic lipids. Significantly different from control incubations: * $P<0.01$, ** $P<0.001$.

	^{3}H(d.p.m.) in:					
	InsP		InsP$_2$		InsP$_3$	
Time	Control	+[Arg^8]vasopressin	Control	+[Arg^8]vasopressin	Control	+[Arg^8]vasopressin
15s	5850 ± 660 (15)	6900 ± 880 (15)	2010 ± 200 (15)	4350** ± 380 (15)	470 ± 45 (15)	1520** ± 155 (15)
60s	15200 ± 1710 (23)	13500 ± 1170 (28)	7750 ± 880 (23)	15500** ± 1060 (28)	1460 ± 190 (23)	3390* ± 310 (28)
10min	13900 ± 1060 (9)	54300** ± 2490 (11)	8470 ± 1110 (9)	47200** ± 1990 (11)	860 ± 90 (9)	7250** ± 420 (11)

Table 3. *Inhibition by a V_1-vasopressin antagonist of inositol phosphate accumulation induced by [Arg^8]vasopressin in superior cervical ganglia*

Ganglia were incubated as described in the Materials and methods section. [1-(β-Mercapto-β,β-cyclopentamethylenepropionic acid), 8-arginine]vasopressin was present together with 10mM-LiCl for 5min before [Arg^8]vasopressin was added for a further 10min. Results are means ± S.E.M. (numbers of ganglia analysed in parentheses).

Conditions	^{3}H in inositol phosphates (d.p.m./10^5 d.p.m. in lipid)
Control	4860 ± 550 (5)
[Arg^8]vasopressin (6.48×10^{-8}M)	43700 ± 1370 (5)
[Arg^8]vasopressin (6.48×10^{-8}M) + V_1-antagonist (10^{-6}M)	5540 ± 1470 (5)

'gates'). These are circumstances under which there should be no release of intrinsic neurotransmitters within the ganglia. Under these conditions there was a substantial decrease in the amounts of inositol phosphates observed in the control ganglia, possibly reflecting a decrease in the Ca^{2+}-dependent spontaneous activity of the ganglionic neurones, but a large response to externally added vasopressin was still observed (Table 4).

Effects of adrenaline and vasopressin on cyclic AMP levels

The results reported above clearly indicate that rat superior cervical ganglia contain a functionally active population of vasopressin receptors of the V_1-type. However, they give no indication as to whether or not functional V_2-receptors, which cause activation of adenylate cyclase, are present. The data presented in Table 5 show that adrenaline stimulates a large accumulation of cyclic AMP, as has been reported previously (e.g. Greengard & Kebabian, 1974). By contrast, 0.64 μM-[Arg^8]vasopressin produced no significant change in ganglionic cyclic AMP levels. This suggests that these ganglia have few, if any, V_2-vasopressin receptors coupled to adenylate cyclase.

Discussion

It has been known for many years that muscarinic cholinergic stimuli cause an increase in inositol lipid turnover in isolated sympathetic ganglia and that this response occurs either exclusively or primarily in the major noradrenergic neuronal cell bodies that constitute much of the ganglionic mass (Hokin *et al.*, 1960; Hokin, 1965; Lapetina *et al.*, 1976; Pickard *et al.*, 1977). Although it has generally been assumed that this

Table 4. *Lack of dependence of [Arg^8]vasopressin-induced inositol phosphate accumulation upon Ca^{2+} in the extracellular medium*

Isolated ganglia were labelled with [2-^{3}H]inositol as described in the text. Incubations were performed in the presence or absence of Ca^{2+} (0.9mM) and nifedipine (10μM). Following a 5min incubation with 10mM-LiCl the ganglia were stimulated with [Arg^8]vasopressin (0.32μM) for 10min in the appropriate medium. The values represent the means ± S.E.M. of the combined inositol phosphates corrected to an incorporation of 10^5 d.p.m. into ganglionic lipids from five separate incubations.

Conditions	^{3}H in inositol phosphates (d.p.m./10^5 d.p.m. in lipid)		Increase (%) induced by [Arg^8]vasopressin
	Control	+[Arg^8]vasopressin (0.32μM)	
Ca^{2+} (0.9mM)	20200 ± 4800	65100 ± 7640	323
Ca^{2+}-free	12300 ± 3600	43900 ± 9400	357
Ca^{2+} (0.9mM) and nifedipine (10μM)	8040 ± 1360	55900 ± 11400	695
Ca^{2+}-free and nifedipine (10μM)	5300 ± 1650	29900 ± 5130	564

Table 5. *Effects of adrenaline and [Arg^8]vasopressin on the cyclic AMP content of rat superior cervical ganglia*

Ganglia were excised and incubated in the presence of 10mM-theophylline for 10min to inhibit cyclic nucleotide phosphodiesterases. Adrenaline (0.1mM) or [Arg^8]vasopressin (0.64μM) was then added to the medium. Incubations were terminated after 20min and cyclic AMP was assayed as described in the text. Results are means ± S.E.M. for nine separate ganglia.

Conditions	Cyclic AMP (pmol/ganglion)
Control	0.85 ± 0.08
0.1mM-Adrenaline	16.70 ± 1.35
0.64μM-[Arg^8]vasopressin	1.18 ± 0.19

response, like receptor-mediated stimulation of inositol lipid metabolism in other tissues, involves the stimulated hydrolysis of inositol lipid(s) by a phosphodiesterase, the data presented in Table 1 constitute the first direct evidence in support of this view. It is apparent that during 60min of sustained muscarinic cholinergic stimulation, the accumulation of labelled inositol phosphates was equivalent to about one-quarter of the labelled inositol lipids initially present in the ganglia. About one-fifth of these accumulated inositol phosphates were in the form of InsP_2 or InsP_3, indicating that at least this fraction of the initial response took the form of a breakdown of either PtdIns4P or PtdIns4,5P_2; the main accumulation of Ins$P$ could have been a result either of a primary breakdown of PtdIns or of polyphosphoinositide hydrolysis followed by phosphomonoesterase attack upon the released phosphate esters.

The most striking result reported here is that vasopressin stimulates inositol lipid metabolism in these ganglia to a much greater degree than does muscarinic cholinergic stimulation. The response to vasopressin can be detected easily in 15s (Table 2), is sustained for at least 1h (Fig. 1) and involves the generation of inositol phosphates at about six times the rate seen with bethanechol. As a result, ganglia incubated with vasopressin for 1h accumulate approx. 1.5×10^5 d.p.m. in inositol phosphates, even though at any time during the incubations they only have about 10^5 d.p.m. in their inositol lipids. They must therefore be both degrading and resynthesizing 2–3% of their [^{3}H]-inositol-labelled lipids every minute. Despite this rapid drain on the inositol lipid pool, the total amount of labelled lipids in the ganglia changes little during stimulation, suggesting that, despite the attempted use of a pulse–chase experimental design, the ganglia retain a substantial store of labelled inositol that remains available for lipid synthesis. The present results might equally be explained either by the turnover of the entire labelled inositol lipid pool of the vasopressin-stimulated ganglia about once every 40min or by the turnover of a more highly labelled, but smaller, lipid pool every few minutes.

After 15 or 60s of vasopressin stimulation the only accumulated products were InsP_2 and InsP_3 (Table 2), so the initial response to stimulation must include phosphodiesterase attack upon PtdIns4,5P. The InsP_2 could come either from the liberated InsP_3 or directly from hydrolysis of PtdIns4P. The large accumulation of Ins$P$ during longer periods of stimulation in the presence of Li^+ seems likely to be derived from breakdown of InsP_2 and InsP_3 rather than from direct phosphodiesterase attack upon PtdIns.

Accumulation of inositol phosphates is seen in ganglia incubated under conditions designed to minimize the endogenous release of neurotransmitters. This suggests that stimulation of polyphosphoinositide phosphodiesterase is a direct response to vasopressin stimulation rather than a secondary consequence of the release of another neurotransmitter within the ganglia.

As in other tissues [liver (Kirk *et al.*, 1979, 1981*b*; Michell *et al.*, 1979; Creba *et al.*, 1983), arterial smooth muscle (Takhar & Kirk, 1981) and hippocampus (L. Stephens & S. Logan, personal communication)], stimulation of inositol lipid metabolism in ganglia by vasopressin involves receptors of the V_1-type. The inability of vasopressin to increase ganglionic cyclic AMP concentrations suggests that this tissue possesses few, if any, functional vasopressin receptors of the V_2-type.

We do not yet know which cells within the stimulated ganglia show this dramatic biochemical response to V_1-vasopressin stimulation, but its magnitude might suggest that it can be assigned to the major cell type, i.e. to the efferent noradrenergic neurones. Nor is there any available information on electrophysiological events, if any, that are evoked or modulated in these cells by stimulation with vasopressin. However, the striking similarity between the V_1-vasopressin-evoked changes in inositol lipid metabolism in sympathetic ganglia, hippocampus and peripheral tissues supports the view (Michell, 1981; Downes, 1982, 1983) that the same primary biochemical coupling events are likely to be involved in the function of these receptors in neurones and elsewhere. Moreover, sympathetic ganglia are much simpler than many of the other nervous tissues in which receptor-activation-stimulated inositol lipid metabolism occurs, so they may afford a better opportunity for elucidating of the role of receptor-activated inositol lipid breakdown in neural function.

A vasopressin-like peptide (VLP) has recently been identified in the major noradrenergic neurones of sympathetic ganglia from several species, including rat, and in nerve fibres innervating tissues that receive substantial sympathetic innervation (Hanley *et al.*, 1984). It appears possible that VLP is a co-transmitter with noradrenaline throughout the sympathetic nervous system, in which case it may be the mediator of many of the peripheral responses to sympathetic stimulation that are resistant to blockade of catecholamine receptors. In addition, it seems possible that many of the vasopressin receptors present in these tissues normally respond to locally released neural 'VLP', rather than to circulating vasopressin of neurohypophyseal origin.

In the original examination of the distribution of VLP within ganglia, it was noted that this material occurred not only in the cell bodies of the principal neurones but also in a plexus of nerve fibres that pervade the ganglia (Hanley *et al.*, 1984). The striking accumulation of inositol phosphates, presumably produced by the breakdown of inositol lipids, reported here as a response of sympathetic ganglia to added vasopressin, initially suggested that there might also be VLP-mediated communication between the individual neurones within the ganglia. However, biochemical studies with K^+-depolarized ganglia suggest that the role of ganglionic receptors is not to respond to locally released VLP (E. A. Bone & R. H. Michell, unpublished work).

Note added in proof (Received 30 May 1984)

Since submission of this paper, we have learned that vasopressin inhibits transmission through sympathetic ganglia (Wali, 1984) and produces a slow excitation of some ganglionic neurones (Peters & Kreulen, 1984; S. Logan, personal communication). It seems possible that the presence of a large population of V_1-receptors in sympathetic ganglia allows circulating hormonal vasopressin to influence VLP-mediated neurotransmission in the sympathetic nervous system (Michell *et al.*, 1984*a*).

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