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# Discrimination between the functional and biochemical effects of two herbal oxytocics on the rat myometrium

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**Abstract**

This study on the rat myometrium is the first report where the effects of herbal extracts used as oxytocics in traditional medicine have been systematically analysed in the same preparation at the level of functional (contractile) and biochemical (second messenger generation) responses. Extracts of *Agapanthus africanus* and *Clivia miniata* (used in South African traditional medicine) were compared with other uterotonic agents with regard to their ability to stimulate phosphoinositide metabolism in the rat myometrium and cause accumulation of [$^3$H]inositol phosphates. The maximal contractile response of the isolated rat myometrium in response to stimulation by the herbal extracts and agonists was compared with the maximal contractile response to cumulative addition of acetylcholine. The rank order of intensity of stimulation of [$^3$H]inositol phosphate generation was: oxytocin > *Agapanthus* > prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) > serotonin > acetylcholine > *Clivia* > ergometrine. This differed from the rank order of maximum contractile response: oxytocin > acetylcholine > $PGF_{2\alpha}$ > serotonin ≈ *Clivia* > *Agapanthus* > ergometrine. Activity was also identified in chemical fractions of the plants and components common to both plants have been identified in the isolated active fractions. These results have identified that the uterotonic activity of *Agapanthus* is linked to increased turnover of phosphoinositides as a signal transduction mechanism, whereas this appears to play a less significant role in the uterotonic activity of *Clivia*. This study illustrates the benefits of using the measurement of stimulation of phosphoinositide metabolism as a bioassay in phytomedical research.

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## Introduction

The oxytocic agents approved for clinical use in obstetrics to induce or augment labour or control post-partum bleeding are: oxytocin, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), prostaglandin $E_2$ and ergometrine. The effects of these drugs on contraction of the pregnant human uterus have been well documented. However, in many third world countries, particularly in rural areas, traditional herbal remedies are often used to induce or augment labour. Decoctions of the leaves of *Agapanthus africanus* (uhlakahla, ubani) and *Clivia miniata* (umayime) are used as oxytocic agents in South African traditional herbal medicine (Veale et al 1992). Aqueous extracts of *C. miniata* and *A. africanus* leaves have been shown to possess uterotonic activity in the isolated rat whole uterus preparation (Veale et al 1989, 1999; Veale 1992) and in the stripped myometrium preparation (Veale et al 2000).

It is well established in various types of smooth muscle, including myometrium, that contractile activity is determined by the level of intracellular free $Ca^{2+}$. $Ca^{2+}$

mobilization from intracellular sources has been described to result from the activation of cell surface receptors which are coupled to the hydrolysis of phosphoinositides by phospholipase C. The degradation of phosphatidylinositol 4,5-biphosphate leads to the formation and release of two intracellular messengers, inositol triphosphate ($IP_3$) and diacylglycerol, into the cell cytoplasm. $IP_3$ stimulates the release of stored intracellular $Ca^{2+}$ from $IP_3$-sensitive pools in the sarcoplasmic reticulum, as well as stimulating the influx of extracellular $Ca^{2+}$ through membrane $Ca^{2+}$ channels, thus increasing free cytosolic $Ca^{2+}$ (Phillippe 1994). Diacylglycerol activates $Ca^{2+}$/phospholipid-dependent protein kinase C, which is reported to play an important role in the agonist-induced contraction of the uterus by modulating the activity of voltage-dependent $Ca^{2+}$ channels in myometrial smooth muscle cells (Shimamura et al 1994).

It has been shown that the uterotonic action of oxytocin, $PGF_{2\alpha}$, and agonists acting on $M_3$ muscarinic cholinergic receptors and $\alpha_1$-adrenoceptors is mediated by the activation of phospholipase C, resulting in the accumulation of $IP_3$ and diacylglycerol (Marc et al 1986; Ruzycky & Triggle 1987; Varol et al 1989; Coleman et al 1994).

The aim of this study was to determine whether the uterotonic activity of *Clivia* and *Agapanthus* could also be linked to the activation of phospholipase C and stimulation of phosphoinositide metabolism as a signal transduction mechanism, and to relate the level of activity to that of clinically used oxytocic agents. The herbal extracts and a series of uterotonic agonists were therefore examined for their ability to stimulate the formation of [$^3$H]-labelled inositol phosphates in rat myometrial strips. The maximal contractile response of the isolated rat myometrium to uterotonic agonists and the herbal extracts was also examined to determine a possible correlation between contractile activity and the stimulation of phosphoinositide metabolism.

## Materials and Methods

### Materials

Acetylcholine chloride (Sigma); ergometrine maleate (Sigma); serotonin creatinine sulfate monohydrate (5-HT) (Aldrich/Sigma); oxytocin (Syntocinon, Novartis); $PGF_{2\alpha}$, (Prostin $F_{2\alpha}$; Pharmacia Upjohn); lithium chloride (Saarchem); stilboestrol (Maybaker) diluted in arachis oil; myo-[2-$^3$H]inositol (Amersham); Dowex AG1-X8 formate form, 200–400 mesh (Bio-Rad); Ultima Gold scintillant (Packard).

### Plant material and preparation of the extracts

Authenticated *A. africanus* (L.) Hoffmg. (family Alliaceae) and *Clivia miniata* (Lindl.) Regel (family Amaryllidaceae) plants were cultivated in a garden in Sandton (Gauteng, South Africa) to ensure a controlled source of plant material. The plants were authenticated and voucher specimens prepared by Ms R. Reddy (C. E. Moss Herbarium, University of the Witwatersrand; voucher nos. H2H45 (*A. africanus*) and J85052 (*C. miniata*)).

Leaves were washed, dried, cut into small pieces, weighed and then simmered in de-ionized water for 30 min. The extraction procedure closely resembled the ethnic method of preparation. The aqueous filtrate was lyophilized and weighed and the extract yield was calculated relative to the wet starting material (2.6%, w/w, *A. africanus* and 0.8%, w/w, *C. miniata*). The lyophilized crude extract was resolubilized in distilled water or Krebs Ringer bicarbonate buffer (KRB) to the required concentrations. The aqueous herbal extracts were therefore evaluated as herbal medicines according to the World Health Organization definition (World Health Organization 1996).

Ethanolic extractions were also made of the fresh leaves of *A. africanus* and *C. miniata*. These extracts were filtered and acidified to pH 3. A 20-mL sample of each extract was applied to a 50-g silica gel G-HR (TLC grade; Machery Nagel) column using the vacuum liquid chromatography method of Pelletier et al (1986), and gradient elution with chloroform, acetone and methanol was carried out. The fractions were collected and the solvents evaporated off. The residues were resolubilized in 1 mL ethanol and dilutions were made such that the tissue samples were not exposed to concentrations of ethanol $> 1\%$.

### Chromatography

A comparative liquid chromatographic analysis was performed on the ethanolic whole leaf extracts and active acetone fractions to assess the relative content of some of the constituents. A Hewlett Packard 1090 HPLC system was used with a ternary gradient pump, autosampler, diode array UV detector and HP Chemstation (ver. 06.04.02) software. A $C_{18}$ reversed-phase column was used for the separation (Luna $C_{18}$(2), 5 $\mu$m, 250 × 4.6 mm; Phenomenex, Torrance, CA), with gradient elution from 0% acetonitrile to 50% acetonitrile in water after 15 min. The flow rate was 1 mL min$^{-1}$ with UV detection at 205 nm, and an injection volume of 5 $\mu$L.

### Tissue preparation

Virgin female Sprague-Dawley rats (300 g) were injected with stilboestrol in arachis oil (0.1 mg $kg^{-1}$, i.p.) and were then killed by inhalation of $CO_2$ 24 h later. Ethical approval was granted by the Animal Ethics Committee of the University of the Witwatersrand (Clearance certificate no. AEC 97/5/3) in accordance with international standards. The uterine horns were dissected out and opened longitudinally and the myometrium was prepared free of endometrium (Hodgson & Daniel 1973; Veale et al 2000).

### Measurement of [$^3$H]inositol phosphates

The method of Marc et al (1986) was followed with modifications. Initially, the individual response to acetylcholine by strips of whole uterus, myometrium and endometrium was determined. The endometrium, obtained by the stripping of the myometrium, contained some circular muscle (Hodgson & Daniel 1973). Thereafter, only myometrial strips were used.

Strips of tissue (50–100 mg) were placed in 5 mL KRB (pH 7.4) after dissection and were then blotted and weighed. A tissue strip from the matching uterine horn of each rat was used as a basal control in every experiment. Each strip was then individually incubated with 12 $\mu$Ci of myo-[2-$^3$H]inositol in 1 mL KRB buffer in a shaking water bath at 30°C (gas phase 95% $O_2$ plus 5% $CO_2$). Incubation continued for 3.5 h by which time the incorporation of [$^3$H] into inositol lipids should have reached a plateau (Marc et al 1986).

The tissue strips were then rinsed 3 times with 20 mL KRB, and transferred to 1.5 mL fresh buffer and allowed to equilibrate for 20 min before transfer to 1.0 mL fresh buffer containing 10 mM lithium chloride. Lithium was added to inhibit inositol monophosphatase in order to enhance the accumulation of the inositol phosphates and prevent metabolism to inositol.

After 10 min, the tissue was transferred to fresh buffer containing either 10 mM lithium chloride alone for the control strips, or the specific concentration of agonist to be tested. Incubation was continued for a further 10 min. Reactions were stopped by immersing the tissue in 1 mL ice-cold 10% (w/v) trichloroacetic acid (TCA) and followed by snap-freezing in liquid nitrogen and storage at −70°C. Each frozen strip was pulverized in liquid nitrogen, followed by homogenization (Ultra-Turrax with N10 shaft; Janke & Kunkel) in 1.5 mL 10% TCA on ice and centrifugation at 10000 *g*, at 4°C for 10 min. The TCA supernatants which contained the water-soluble inositol metabolites were extracted 4 times with 4 mL diethyl ether to remove the TCA. The extract was then neutralized with KOH and was applied to a column (1 × 2.5 cm) of Dowex AG1-X8 formate form, 200–400 mesh.

Free inositol was eluted by 10 mL de-ionized water and glycerophosphoinositol was eluted by 10 mL 5 mM sodium tetraborate and 60 mM ammonium formate. Inositol monophosphate ($IP_1$), inositol biphosphate ($IP_2$) and $IP_3$ were then successively eluted by: 10 mL 0.1 M formic acid and 0.2 M ammonium formate; 10 mL 0.1 M formic acid and 0.5 M ammonium formate; and 10 mL 0.1 M formic acid and 1 M ammonium formate. Each peak was collected as a single fraction and 2.5 mL of each fraction was taken for determination of radioactivity in scintillation fluid. Results are expressed as counts $min^{-1}$ (mg tissue)$^{-1}$ or as a percentage of total inositol phosphate accumulation compared with control.

### Measurement of myometrial contractile responses

The method of Veale et al (2000) was followed. Myometrial strips were placed in 50-mL jacketed organ baths filled with Tyrode solution aerated with 5% $CO_2$ in oxygen. The bath temperature was maintained at 26°C and isotonic contractions against a load of 1 g were recorded electronically. The myometrial strips were allowed to equilibrate for a period of approximately 30 min and then cumulative dose–response curves were constructed at 30-min intervals using acetylcholine as a standard agonist. When the concentration–response curve to acetylcholine stabilized (usually after 3 consecutive curves ± 3 h later) the uterotonic agonist or plant extract was administered cumulatively until maximal response was attained. Each test curve was then followed by another acetylcholine control curve. The organs were allowed to rest for at least 30 min between drug challenges and the organ baths were well rinsed during this period. Matching uterine horns were used as time-matched controls.

### Statistical analysis

Data are expressed as means ± s.e.m., and n refers to the number of rats. The significance of differences between two means was evaluated by the Student's *t*-test using GraphPad Instat 2. Graphs were generated using GraphPad Prism (ver. 1.0).

## Results

This study used the measurement of [$^3$H]inositol phosphates as an index of phosphoinositide hydrolysis in rat myometrium. Initial experiments comparing whole uterus, myometrium and endometrium indicated that the myometrial preparation was significantly more sensitive to acetylcholine stimulation of phosphoinositide hydrolysis than the whole uterus preparation (Table 1). The myometrium was therefore used as the standard preparation in all further experiments to eliminate the effects of spontaneous release of prostaglandins from the endometrium (Veale et al 2000). Acetylcholine has proved to be a very reliable standard agonist in the isolated stripped myometrium preparation, capable of reproducible results and not inducing spontaneous contractions or sensitizing or desensitizing the preparation (Veale et al 2000).

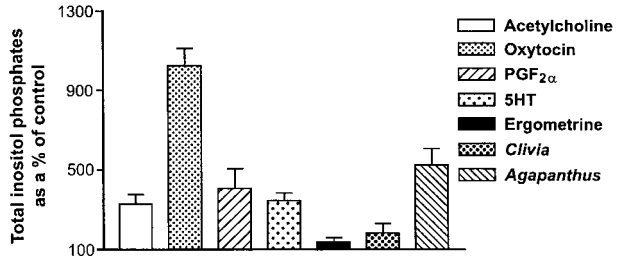


**Figure 1** Total [$^3$H]inositol phosphates accumulated in the rat myometrium in response to stimulation by: acetylcholine (20 mM, n = 8); oxytocin (0.6 $\mu$M, n = 6); $PGF_{2\alpha}$ (70 $\mu$M, n = 5); serotonin (125 $\mu$M, n = 6); ergometrine (1 $\mu$M, n = 5); *Clivia* aqueous extract (40 mg mL$^{-1}$, n = 4) and *Agapanthus* aqueous extract (30 mg mL$^{-1}$, n = 4). All results are expressed as percentage increase over control values from the same experiment. Results are expressed as the mean ± s.e.m., where n represents the number of rats.

**Table 1** The effect of acetylcholine (20 mM) on the accumulation of [$^3$H]inositol phosphates (% of basal) in whole uterus, myometrium and endometrium.

| | Total [$^3$H]inositol phosphates (% of basal) |
|---|---|
| Whole uterus | 215.65 ± 36.48 |
| Myometrium | 528.98 ± 100.23* |
| Endometrium | 258.55 ± 82.26 |

Results are expressed as the mean ± s.e.m., n = 3 rats. Statistical significance was assessed by the Student's *t*-test. The response by myometrial tissue was significantly different compared with whole uterus (*$P < 0.05$).

It was found that the herbal extracts of *A. africanus* and *C. miniata* caused an accumulation of [$^3$H]inositol phosphates, particularly inositol monophosphatase (Table 2) as would be expected after lithium inhibition of $IP_1$. The standard uterotonic agonists, used as control reference agents, also stimulated inositol phosphate accumulation, as has been shown previously (Marc et al 1986; Schrey et al 1988; Molnár & Hertelendy 1990). The rank order of increase in accumulation of total [$^3$H]inositol phosphates above basal due to the indi-

**Table 2** Effect of agonists on the accumulation of [$^3$H]inositol metabolites in rat myometrium (counts min$^{-1}$ (mg tissue)$^{-1}$).

| Treatment | $IP_1$ | $IP_2$ | $IP_3$ | n | Total [$^3$H] inositol phosphates (% of basal) |
|---|---|---|---|---|---|
| Acetylcholine | 430.31 ± 50.88 | 104.37 ± 39.60 | 30.70 ± 4.88 | 8 | 326.90 ± 49.33*** |
| Control | 144.54 ± 10.36 | 22.46 ± 3.45 | 9.45 ± 1.04 | | |
| *Agapanthus* | 544.50 ± 88.78 | 51.08 ± 19.82 | 48.27 ± 8.54 | 4 | 522.27 ± 125.54** |
| Control | 90.83 ± 12.32 | 14.84 ± 4.98 | 10.86 ± 1.55 | | |
| *Clivia* | 144.01 ± 8.20 | 15.15 ± 5.32 | 30.15 ± 2.81 | 4 | 183.42 ± 46.20* |
| Control | 91.78 ± 15.86 | 15.68 ± 6.34 | 11.64 ± 1.72 | | |
| Ergometrine | 163.29 ± 35.88 | 42.73 ± 10.68 | 15.75 ± 2.37 | 5 | 137.43 ± 22.07 |
| Control | 120.75 ± 10.39 | 42.96 ± 12.55 | 11.29 ± 0.42 | | |
| Serotonin | 448.66 ± 100.76 | 218.27 ± 31.86 | 60.15 ± 11.98 | 6 | 344.10 ± 39.74*** |
| Control | 158.10 ± 15.22 | 42.64 ± 3.90 | 14.92 ± 1.34 | | |
| Oxytocin | 804.33 ± 114.17 | 106.49 ± 9.93 | 74.78 ± 14.59 | 6 | 1022.22 ± 89.65*** |
| Control | 77.64 ± 8.55 | 9.01 ± 1.00 | 9.31 ± 0.90 | | |
| $PGF_{2\alpha}$ | 424.14 ± 118.57 | 141.34 ± 53.73 | 28.00 ± 5.97 | 5 | 407.23 ± 97.40* |
| Control | 112.93 ± 18.62 | 26.10 ± 4.73 | 12.80 ± 0.99 | | |

Results are expressed as the mean ± s.e.m., where n represents the number of rats, and the statistical significance (total [$^3$H]inositol phosphate accumulation compared with control tissue) was assessed using the Student's *t*-test (*$P < 0.05$; **$P < 0.005$; ***$P < 0.0005$).

**Table 3** Differences between the stimulation of total [$^3$H]inositol phosphate accumulation (% of basal expressed as a multiple of basal stimulation) and % maximal contractile response relative to maximal contractile response to cumulative addition of acetylcholine in the rat myometrium.

| Stimulation of phosphoinositide metabolism | | | Maximal myometrial contractile response | | |
|---|---|---|---|---|---|
| Treatment | Concn | Fold increase in [IPs] | Bath concn | % Contractile response ± s.e.m. | n |
| Acetylcholine | 20 mM | 3.3-fold | 1 mM | 100.0 | 38 |
| *Agapanthus* | 30 mg mL$^{-1}$ | 5.2-fold | 2.4 mg mL$^{-1}$ | 62.6 ± 6.97 | 29 |
| *Clivia* | 40 mg mL$^{-1}$ | 1.8-fold | 3.2 mg mL$^{-1}$ | 76.3 ± 4.40 | 22 |
| Ergometrine | 1 $\mu$M | 1.4-fold | 1.9 $\mu$M | 49.4 ± 8.12 | 9 |
| Serotonin | 125 $\mu$M | 3.4-fold | 5 $\mu$M | 79.9 ± 6.61 | 38 |
| Oxytocin | 0.6 $\mu$M | 10.2-fold | 0.1 $\mu$M | 110.3 ± 4.69 | 10 |
| $PGF_{2\alpha}$ | 70 $\mu$M | 4.1-fold | 7.9 $\mu$M | 95.0 ± 2.59 | 12 |

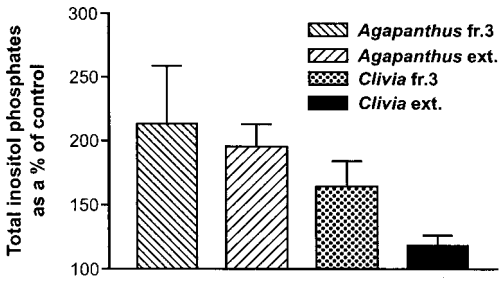


**Figure 2** Total [$^3$H]inositol phosphates accumulated in the rat myometrium in response to stimulation by: *Agapanthus* fraction 3 (n = 6); *Agapanthus* aqueous extract (15 mg mL$^{-1}$, n = 6), *Clivia* fraction 3 (n = 6) and *Clivia* aqueous extract (20 mg mL$^{-1}$, n = 6). All results are expressed as percentage increase over control values from the same experiment. Results are expressed as the mean ± s.e.m., where n represents the number of rats.

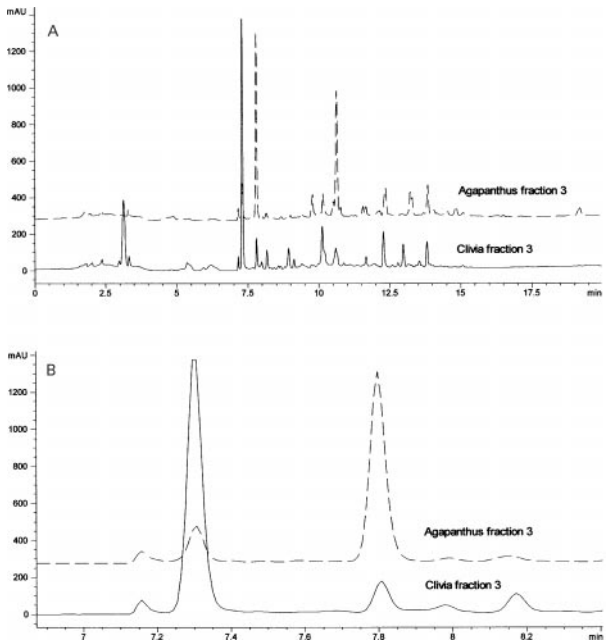


**Figure 3** A. Overlaid HPLC profiles of the constituents in isolated fractions of *Agapanthus africanus* leaf extract and *Clivia miniata* leaf extract. B. Zoomed-in chromatogram of the major peaks in the isolated acetone fractions of *A. africanus* and *C. miniata*.

vidual agents was as follows: oxytocin > *Agapanthus* > $PGF_{2\alpha}$ > serotonin > acetylcholine > *Clivia* > ergometrine (Figure 1). Basal values were measured as the response of a myometrial strip from a matching uterine horn in the absence of agonist or extract.

When the maximal contractile response of the isolated myometrium to cumulative addition of the agonists and aqueous herbal extracts was compared relative to the maximal contractile response of the myometrium to cumulative addition of acetylcholine, the rank order of the response was different: oxytocin > acetylcholine > $PGF_{2\alpha}$ > serotonin ≈ *Clivia* > *Agapanthus* > ergometrine (Table 3). As found by Marc et al (1986), agonists were able to stimulate phosphoinositide hydrolysis at much higher concentrations than those required for maximal activation of contraction in the rat myometrium, indicating that a small elevation of $IP_3$would be sufficient to maximally activate contraction (Table 3). It was noted, however, that ergometrine was an exception; there was no enhancement of phosphoinositide metabolism in response to 100 $\mu$M ergometrine. No correlation was found between the concentration of agonist, measurement of intensity of stimulation of phosphoinositide metabolism and maximal contractile response. An *Agapanthus* concentration 12.5-fold greater than that required for maximal contractile response produced a 5.2-fold increase in total [$^3$H]inosi-

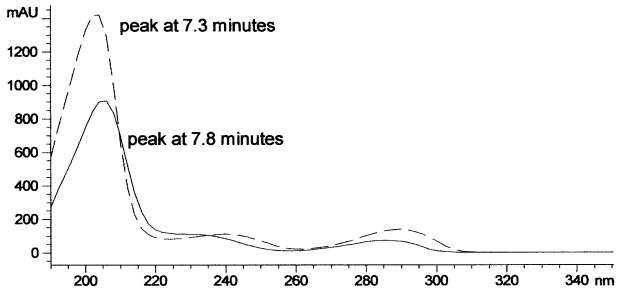


**Figure 4** UV spectra of peaks eluting at 7.3 and 7.8 min, showing the similarity in absorption spectra, suggesting that the peaks may be due to structurally similar compounds.

tol phosphate accumulation, whereas in the case of *Clivia*, the increase was only 1.8-fold (Table 3).

Crude fractionation of the ethanolic extracts of both *Clivia* and *Agapanthus* yielded active fractions (fraction 3, eluted by acetone) able to stimulate phosphoinositide hydrolysis (Figure 2). The *Agapanthus* fraction was more potent than the *Clivia* fraction. *Clivia* fraction 3 and *Agapanthus* fraction 3 also directly stimulated contractile responses in the isolated rat myometrium that were $23.09 \pm 5.08\,\%$ and $21.24 \pm 6.00\,\%$ of the maximal response to acetylcholine, respectively ($n = 4$).

Chromatographic analysis of the active acetone fractions of *Clivia* and *Agapanthus* indicates common peaks at retention times of 7.3 and 7.8 min (Figure 3). Spectral evidence (Figure 4) showed that the fractions most probably contain the same substances, but at different concentrations.

## Discussion

In this study on the rat myometrium the effects of herbal extracts used as oxytocics in traditional medicine have been analysed systematically in the same preparation at the level of functional (contractile) and biochemical (second messenger generation) responses.

Extracts of *A. africanus* and *C. miniata* stimulated phosphoinositide metabolism in the rat myometrium with increased generation of [$^3$H]inositol phosphates. The effect of *Agapanthus* was much greater than that of *Clivia*. Chromatographic analysis of the active fractions of *Clivia* and *Agapanthus* indicate common peaks at retention times of 7.3 and 7.8 min and spectral evidence showed that the fractions most probably contain the same substances, but at different concentrations, and that the compounds may be structurally related. Although the common components cannot be directly related to potency in the active fractions, it is interesting to note that the active *Agapanthus* fraction was more potent than the *Clivia* fraction in this model, as is the case with the crude aqueous extracts. This is in contrast to the contractile activity of the herbal extracts in the stripped myometrium model, where both the crude aqueous extracts and the active fractions show similar activity. These results are the first to identify common chemical components in the active isolated chemical fractions of two uterotonic herbal medicines from different botanical families.

We have already shown that the contractile response of the myometrium to *Agapanthus* is associated with increased prostaglandin synthesis and muscarinic cholinergic activation (Veale et al 2000), and these results appear to confirm the previous findings, that is, response to stimulation by *Agapanthus* activates the same signal transduction pathway as activation of prostanoid receptors and $M_3$ muscarinic cholinergic receptors (Coleman et al 1994; Varol et al 1989).

The effect of *Clivia* on phosphoinositide metabolism was significantly less than its effect on myometrial contraction (Table 3), and the same result was found with ergometrine. The mechanism of activity of *Clivia* has been more difficult to define, and although there appears to be some interaction with muscarinic cholinergic receptors, this plays a minor role in the myometrium (Veale et al 2000). The *Clivia* extract (2 mg $mL^{-1}$) has been shown to act as an antagonist at 5-$HT_2$ receptors in the rat whole uterus preparation, seemingly by a competitive mechanism, that is, by shifting the concentration–response curve of serotonin to the left (Veale 1992). However, *Clivia* appears to act as a partial agonist on 5-$HT_2$ receptors in the stripped myometrium at a concentration of 0.8 mg $mL^{-1}$, causing an initial additive effect and then, at higher concentrations of serotonin, induces a parallel shift of the serotonin concentration curve to the right ($n = 7$). Ergometrine has been shown to have a similar profile of activity on the 5-$HT_2$ receptor in the rat uterus. Hollingsworth et al (1988) showed that ergometrine acted as a partial agonist at lower concentrations (0.1–1 $\mu$M) and a competitive antagonist at higher concentrations (10 $\mu$M). This could indicate allosteric interference on the 5-$HT_2$ receptor and possibly other binding sites, and explain why ergometrine (100 $\mu$M) did not stimulate phosphoinositide metabolism.

This study illustrates that the measurement of [$^3$H]-inositol phosphate accumulation in the rat myometrium is a viable and reproducible bioassay to determine the ability of herbal extracts to activate phospholipase C and the phosphoinositide signal transduction pathway. The advantages of using this bioassay can be sum-

marized as follows: measurement of biochemical activity in the absence of interference from endometrial/decidual secretions such as prostaglandins in studies of uterine activity; indication of activation of $Ca^{2+}$ mobilization from intracellular sources; confirmation of type of transduction mechanism activated by cell surface receptor occupation and correlation to receptor type activated; and the methodology allows for the bioassay of very small quantities of an unknown substance as is often encountered when herbal extracts are subjected to chemical analysis and isolation procedures to identify active principles.

### Conclusion

The present study has shown that extracts and chemically isolated fractions of *A. africanus* and *C. miniata* are able to stimulate phosphoinositide metabolism and that this can be regarded as the signal transduction pathway for the uterotonic activity of *Agapanthus*, as is the case for oxytocin and $PGF_{2\alpha}$. The activity of *Clivia* was less marked in this model and indicates possible additional interactions with other biochemical systems that activate smooth muscle activity. Components common to both plants have been identified in chemically isolated active fractions. The effects of these herbal extracts on $Ca^{2+}$ mobilization in the myometrial smooth muscle cell needs further investigation.

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