**BASIC SCIENCE: OBSTETRICS**

**Methodology and pharmacological analysis of effects of uterotonic compounds in human myometrium in vitro**

Denis J. Crankshaw, PhD; John J. Morrison, MD, FRCOG

**OBJECTIVE:** The methodology used to evaluate contractile effects of uterotonic agents in human myometrium in vitro varies. The are no studies evaluating the reliability of these commonly used techniques.

**STUDY DESIGN:** Myometrial strips (n = 72) were exposed to 3 known uterotonic agents: oxytocin, U46619, and phenylephrine. The negative log of the molar concentration of the agonist that produces a half-maximal response (pEC\(_{50}\)) and maximal response values were obtained, and compared, when either amplitude or mean force was used as indices of contraction. All data were expressed as a percentage of KCl elicited activity.

**RESULTS:** Using pEC\(_{50}\) measurements, the order of potency was oxytocin greater than U46619 greater than phenylephrine for both indices, whereas the order of maximal response varied between mean force and amplitude. The coefficient of variation was lowest for pEC\(_{50}\) measurements, highest for maximal force estimations, and overall was 10-48% between, and 2-27% within, donor samples.

**CONCLUSION:** These findings support the use of pEC\(_{50}\) measurements for in vitro experiments using uterotonic agents and outline the variability that occurs for such myometrial experiments.

**Key words:** human myometrium, maximal response, oxytocin, phenylephrine, U46619, uterotonic agents

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Research has focused on factors modulating human uterine contractility during pregnancy, and labor is important in terms of understanding the normal physiology of such events and also for evaluating novel therapeutic interventions for pathophysiological processes such as preterm labor, dysfunctional uterine activity, and postpartum hemorrhage. These clinical problems are associated with significant perinatal, and maternal morbidity and mortality, in clinical practice.\(^2\) The use of in vitro pharmacological techniques for investigating human uterine contractility has become more widespread in recent years, and, unlike previously used clinical tocoographic technology, the in vitro approach allows for objective assessment of contractile performance and greater understanding of the potential effects of inhibitory and uterotonic compounds.\(^5\)

In addition, technological advances in the design of modern data acquisition systems, and their associated software products, have enabled the researcher to make many different measures of uterine contractility over a period of time. These measurements include frequency of contractions, amplitude, maximum force of contractions, average force of contractions, integrals of contractile area under the curve, and the proportions of any measure of contractile activity observed in relation to a known agonist. In practice, most measurements focus on either amplitude or some measure of force (mean contractile force in a period of time, or integral of force).

This myriad of possible measurements leads to confusion in the review process, and there are hitherto minimal data comparing the efficacy or reliability of one measurement vs another. This pertains particularly to compounds that exert a uterotonic effect because the evaluation of the effects of an inhibitory compound can at least be related, by any of the numerous measurements, to the degree of contractile performance observed prior to exposure. However, for drugs that exert a uterotonic effect, evaluation of their effects on contractile activity is more challenging, with no clearly delineated methodology. Different authors use different techniques for assessment of such uterine contractile performance, and none of the techniques have been subjected to scrutiny in terms of objectivity, variability, and reliability.

The aim of this study was to evaluate the effects of 3 different uterotonic agents, oxytocin, the prostanoid TP receptor agonist U46619, and the \(\alpha\)-adrenoceptor agonist phenylephrine, in tissue samples obtained from different patient donors, and within the same patient donor samples, and to investigate some commonly used measurement techniques. First, the use of the negative log of the molar concentration compound which exerts the half maximal effect (pEC\(_{50}\)) as a measurement of the...
sensitivity of both mean contractile force and amplitude of contraction was evaluated. Second, the reliability of the potassium chloride challenge, and its use in the expression of the maximal contractile activity achieved, for both mean contractile force and amplitude were studied. Finally, the variability of results, across tissue samples from different patient donors and from strips obtained from the same patient donors was investigated.

**Materials and Methods**

**Tissue collection**

Biopsy specimens of human myometrial tissue were obtained at elective cesarean section operations carried out at the Department of Obstetrics and Gynecology, University College Hospital Galway, Galway, Ireland. Biopsies were obtained from the myometrium in the midline, in the upper lip of the incision in the lower uterine segment as previously described.6,7 Ethics Committee (institutional review board) approval for tissue collection was obtained from the Research Ethics Committee at University College Hospital Galway, and recruitment of patients was by written informed consent.

The biopsies were placed in physiologic salt solution (PSS) on collection. The PSS contained the following ingredients: 4.7 mmol/L potassium chloride, 118 mmol/L sodium chloride, 1.2 mmol/L magnesium sulphate, 1.2 mmol/L calcium chloride, 1.2 mmol/L potassium phosphate, 25 mmol/L sodium bicarbonate, and 11 mmol/L glucose (Sigma-Aldrich, Dublin, Ireland). Immediately on collection, tissue biopsy specimens were placed in PSS at 4°C. Specimens were transported to the laboratory, transferred to PSS at room temperature, and used for experimentation within 8 hours of initial collection.

**Tissue bath experiments**

The biopsy specimens were dissected free of decidua and serosa, and 8 longitudinal (along the plane of the muscle fibres) myometrial strips were prepared measuring approximately 2 × 2 × 10 mm. The strips were mounted for isometric recording under 20 mN of tension in tissue baths as previously described.6,7 The baths contained 20 mL of PSS, pH 7.4, at a bath temperature of 37°C and gassed continuously with a mixture of 95% oxygen/5% carbon dioxide. When all strips had attained a steady 20 mN baseline, fresh PSS was introduced. Myometrial strips were allowed to equilibrate under these conditions for a period of at least 1 hour, after which they were challenged with potassium chloride (KCl) at a bath concentration of 30 mM. The KCl was left in contact with the tissue for a period of 10 minutes, followed by a PSS washout of the bath for a period of 10 minutes. This was then followed by 2 similar KCl challenges.

Following the washout with PSS after the third KCl challenge, the myometrial strips were exposed to cumulatively increasing concentrations of either oxytocin, the thromboxane A2 mimetic U46619, or the α-adrenoceptor agonist phenylephrine. The initial bath concentration used for each compound was calculated after consideration of the known sensitivities of myometrial smooth muscle to that particular compound from earlier experiments and previous reports.8-10 For all 3 compounds, there were 10 time points, each 12 minutes apart, at which cumulatively increasing doses were added to the tissue bath, each subsequent dose producing an approximate half-log unit increase in bath concentration compared with the previous dose administered. For oxytocin, the initial bath concentration achieved was 1 pmol/L, increasing to a final bath concentration of 44.4 nmol/L. For U44619, the initial bath concentration used was 28.5 pmol/L, increasing to a maximum bath concentration of 1.27 μmol/L. For phenylephrine, the initial bath concentration used was 10 nmol/L, increasing to a final bath concentration of 444 μmol/L.

**Contractile activity measurements**

All contractile activity was simultaneously recorded using the PowerLab hardware unit and the Chart version 4.0 software (AD Instruments, Hastings, UK). The maximum amplitude of contraction (MAMP) in, and the mean contractile force (MCF) for, the latter 10 minutes of the 12 minute exposure to a particular bath concentration of agonist were used as indices of the contractile activity in response to that particular concentration. Data were expressed as a percentage of the third KCl challenge by normalizing to the appropriate MAMP or MCF value.

The measurements were made in a total of 72 myometrial strips, which included 24 following exposure to each of oxytocin, U46619, and phenylephrine, all strips having been dissected from 9 patient donor samples. This allowed for data from all 9 patient donors in relation to all 3 agonists and provided results for 2–3 samples within a single donor (3 agonists among 8 baths) for each agonist. The measurements from the increasing bath concentrations enabled the construction of concentration-effect curves using the Solver Function in Microsoft Excel 2003 (SP3; Microsoft, Redmond, WA) to calculate various parameters of the response as described below.

**Curve fitting and analysis**

In cases in which the response to the agonist was monophasic, concentration-effect curves were constructed from the data obtained and fitted to a 1 receptor model using the following equation:

\[ E = E_{\text{max}}/(1 + (10^{\text{EC}_{50}} \times \text{pEC}_{50})) \]  

where \( E \) is the effect of the agonist, \( C \) is the molar concentration of the agonist, and \( \text{pEC}_{50} \) is the negative log of the molar concentration of the agonist that produces a half-maximal response.

In cases in which the response appeared biphasic, an attempt was made to fit concentration-effect curves to a 2 receptor model using the following equation:

\[ E = [E_{\text{max}}/(1 + (10^{\text{EC}_{50}} \times \text{pEC}_{50}))] + [E_{\text{min}}/(1 + (10^{\text{EC}_{2}} \times \text{pEC}_{2}))] \]

where subscripts 1 and 2 represent the 2 opposing receptor populations. Equation 2 is derived from equation 1 using the model of Szabadi.11 The applicability of the 1 or 2 site model was assessed using the F test.12 After curve fitting, average values for each patient sample were obtained for the following parameters: \( \text{pEC}_{50} \) for
MCF, pEC$_{50}$ for MAMP, maximum MCF and maximum MAMP values observed. This allowed us to compare MCF and MAMP as indices of both sensitivity (pEC$_{50}$) and contractility (E$_{max}$) of the myometrium to the 3 different agonists, across and within patient donor samples. Comparisons between the values obtained were performed using a Student t test with Bonferroni correction for multiple comparisons. Values of $P < .05$ were considered significant. Variation of results across and within tissue samples was evaluated by measuring the coefficient of variation percentage (CV%) according to equation 3:

$$CV\% = \frac{SD}{Mean} \times 100 \quad (3)$$

where SD is the standard deviation of the mean.

**RESULTS**

Myometrial biopsy specimens were obtained from 9 patients undergoing cesarean delivery for the following reasons: breech presentation, n = 2; suspected cephalopelvic disproportion, n = 2; previous cesarean section, n = 5. The mean age of the patients was 36.1 years (range, 34 –39) with median parity of 1 (range, 0 –2). The median gestation at delivery was 39 weeks (range, 38 –41).

In Figure 1, A-C, representative recordings of the entire contractile activity throughout an individual experiment are shown. The 3 recordings in this figure were obtained from myometrium from a single patient donor. In Figure 2, A-F, the corresponding concentration-effect curves constructed from the data from these recordings for both MCF and MAMP are demonstrated. The concentration effect curves for oxytocin and U46619 (Figure 2, A-D) were always best fitted to equation 1, indicating a monophasic response, whereas the concentration effect curves for phenylephrine were frequently best fitted to equation 2 as demonstrated in Figure 2, E, indicating a biphasic response.

Of the 9 biopsy specimens studied, 6 gave full concentration-response curves to oxytocin. In 2 specimens, the maximum response was not attained over the concentration range used, whereas the remaining biopsy had robust spontaneous contractions that were unaffected by any of the agonists. Seven of the biopsies yielded full concentration-response curves to U46619. In 1 biopsy, the response to U46619 reached its maximum after the first dose.

Concentration-response curves to phenylephrine were obtained from all 8 responsive specimens. For 18 of 22 strips, the MCF showed a significantly biphasic dependence on the phenylephrine concentration, whereas for MAMP, only 7 of 22 strips were significantly biphasic.

The means of the average pEC$_{50}$ values for all specimens showing inhibition by phenylephrine were 4.7 ± 0.3 (n = 8) for MCF and 4.9 ± 0.2 (n = 4) for MAMP. A summary of the contractility parameters for the excitatory component of the responses is given in Table 1.

In Table 1, the average pEC$_{50}$ and maximum response values for both MCF and MAMP across all donors are shown for each agonist separately. The SE for each mean is also shown.

In Table 2, the coefficients of variation values are shown for the various measurements, between donor tissue sam-
Comparison of mean pEC\textsubscript{50} values (Table 1) revealed the order of potency to be oxytocin greater than U46619 greater than phenylephrine for both MCF and MAMP measurements.

It is evident from Table 2 that there is considerable variation across samples in all of the measurements made. The coefficient of variation range for between-donor measurements was from 10% to 48%, as shown in Table 2. The coefficients of variation were highest for MCF, lowest for pEC\textsubscript{50}, and intermediate for MAMP measurements. In contrast, within the same patient donor samples, the coefficients of variation were of a much lesser magnitude, ranging from 2% to 27% with the same order (Table 2).

**COMMENT**

The data from this study support the methodological approach of using pEC\textsubscript{50} values to assess the sensitivity of human myometrial tissue to a uterotonic agent. Second, the results presented in this article provide comparative data for other agonists and pEC\textsubscript{50} calculations.

There are, however, some noteworthy observations. The pEC\textsubscript{50} values measured were lower in magnitude when MAMP was used as the index of contractility than when MCF was the index (ie, of the 3 agonists examined), the amplitude of contraction was more sensitive to the uterotonic effect of the agonist than the mean contractile force developed.

The potency order of the 3 agonists was as follows: oxytocin was greater than U46619 was greater than phenylephrine and was not affected by the choice of index. Additionally, in relation to variability in results obtained between different strips used for the experiments, either from different patient donors or within the same donor, the pEC\textsubscript{50} calculations displayed the lowest variability. This relatively low coefficient of variation ap-
plied similarly to pEC$_{50}$ calculations based on MCF or MAMP.

The KCl challenge is frequently used for the purposes of obtaining a baseline reference for contractile activity, allowing maximal measures of contractility (amplitude or force) to be later expressed as a proportion of this standard. The KCl challenge remains useful to this end and is also independent of receptor-mediated effects. There are also circumstances in which pEC$_{50}$ measurements are less important, namely when it is desirable to know the maximum uterine effect a compound exerts in myometrium.

When expressing the effects of a uterotonic agent as a proportion of a KCl challenge, the results obtained differ markedly, depending on whether mean contractile force or amplitude are the primary measures of contractility used. The maximum response observed with these 3 agonists when MCF was used was in the range of 37-48% of that measured for a similar time period after the KCl challenge, whereas the maximum response observed when MAMP was used were in the region of 132-163%.

As can be seen in Figure 1, this can be explained by the tonic nature of the response to KCl compared with the phasic response to the uterotonic agonists. However, the order of maximum response when MCF was used as the index of contractility was oxytocin = U46619 = phenylephrine, whereas that observed when MAMP was the index was U46619 = oxytocin greater than phenylephrine. The ability of MAMP to discriminate between agonists based on their maximal responses may be linked to the fact that maximum MAMP responses were subject to less variation than the maximum MCF responses. Thus, the coefficient of variation values measured were of the order of 22-24% for maximal MAMP for strips from different donors and were 27-48% for maximal MCF for strips from different donors.

Taken together, all of these findings suggest that comparison of the maximal effect of a uterotonic agent in vitro displays a wide variation in the results obtained and hence requires a larger number of experiments to make meaningful conclusions, particularly when the measurement is of mean contractile force.

The coefficient of variation measurements between patient donors of myometrial tissue samples, and within the same patient donor, provide interesting information for interpretation of in vitro experiments. For all 3 agonists, and for all measurements (ie, pEC$_{50}$ values and maximal effect using both MCF and MAMP as indices), there was a marked apparent reduction in variability in results obtained from experiments on strips excised from the same biopsy. This matter has not hitherto been investigated, and these findings indicate that there may well be significant variation in vivo of the sensitivity of the myometrium to agonists that are used in clinical practice, such as oxytocin.

These results also raise the possibility that another confounding variable in the interpretation of results from in vitro recordings is the morphological structure of the strip itself and particularly the ratio of smooth muscle to connective tissue. There are no data to our knowledge on this topic.

There are some limitations to the design of the experiments and the interpretation of the findings. First, the model outlined did not address frequency of contractions as a separate parameter. For the time period of investigation, the maximum amplitude, or the mean contractile force, was measured because they

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<th>TABLE 1</th>
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<td><strong>Contractility parameters for excitatory agonists on human pregnant myometrium</strong></td>
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Maximum values are expressed as a percentage of the response to the third potassium chloride challenge. Data are shown as means ± SEM from 6-9 observations, each of which is the average of triplicate or duplicate determinations from the same biopsy specimen. Values with the same superscript are significantly different from each other, paired t test. MAMP, maximum amplitude of contraction; MCF, mean contractile force; pEC$_{50}$, half maximal effect.

$^a,b,c$ Unpaired Student t test with correction for multiple comparisons; $^d,e$ P < .05 in all cases.


**TABLE 2**

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<th><strong>Variation in estimates of contractility parameters between and within donors</strong></th>
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<td><strong>Agonist</strong></td>
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Variation is shown as CV%. For comparisons between donors, values are for the overall data shown in Table 1. For comparisons within donors, CV% values were determined for each experiment in which 3 strips from the same donor were used (n = 4-6), and the data shown are means ± SEM from those experiments.

CV%, coefficient of variation percentage; MAMP, maximum amplitude of contraction; MCF, mean contractile force; pEC$_{50}$, half maximal effect.

were deemed to be highly representative of the contractile activity. Although little is known about the factors that regulate spontaneous frequency, it appears to be very similar over time in in vitro experiments. Measurements of frequency are also complicated by the fact that for standard time periods used in such experiments (ie, 10-20 minutes), the frequency may vary, depending on where a contraction starts and finishes in that time; hence, direct comparisons are difficult.

Second, the model used does not account for the possibility that there may be altered fiber orientation in a myometrial strip, which may influence the overall force measured. However, if this were the case, it would have applied similarly to the results from all experiments. This is an important topic for further investigation.

Finally, it is possible, albeit not tested to our knowledge, that length-tension relationships may vary from strip to strip. In such a case, there might be less variability in maximal force generation between samples if each strip was tested at its own optimal tension. For the hypothesis addressed in the current study, we used a standard resting tension because optimizing individual strips is a time-consuming procedure that would likely preclude further experimentation as a result of tachyphalaxis or fatigue.

In summary, this study has focused in detail on the pharmacological measurements made from standard in vitro myometrial recordings in relation to the effects of uterotonic drugs. These findings indicate that pEC50 determinations display lesser variability, are consistent between indices of measurement, and allow for comparable results across different agonists. In contrast, for determination of maximal responses relative to a standard such as a KCl challenge, the values obtained have to be interpreted relative to that standard; vary significantly, depending on whether it is MCF or MAMP that is determined; and, finally, show a relatively large variation within themselves (ie, force or amplitude).

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REFERENCES