COMPARISON OF EFFECTS OF EXTERNAL Ca²⁺ ON ANTI-OXYTOCIN ACTIONS OF ANTISPASMODICS IN OVARIECTOMIZED AND ESTRADIOL-TREATED RAT UTERI

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The influence of Ca²⁺ on the antioxytocin actions of papaverine, deoxycholate and 1,1-diphenyl-3-piperidinobutanol hydrochloride (Aspaminol) was investigated in the ovariectomized and the estradiol-treated rat uterus, and Ca exchangeability and Ca content in both uteri were compared. No qualitative difference in the responses to deoxycholate and Aspaminol at normal and higher Ca²⁺ concentrations in the estradiol-treated rat uterus was seen as compared to those obtained from the ovariectomized one. The antioxytocin action of papaverine in the estrogenized uterus was antagonized by excess external Ca²⁺, but not in the ovariectomized one. There were no significant differences either in the total Ca content or the non-displaceable Ca by La between the ovariectomized and the estrogenized rat uterine longitudinal muscle layers, while Ca exchangeability in the estrogenized specimen was much greater than that in the ovariectomized one. These results suggest that the antagonism of papaverine against excess exogenous Ca²⁺ may be related to the more increased Ca exchangeability in the estradiol-treated rat myometrium.

Keywords — rat uterus; papaverine; deoxycholate; 1,1-diphenyl-3-piperidinobutanol; external Ca²⁺; Ca content; Ca exchangeability

It is well known that the responses of a rat uterus to sympathomimetic amines vary with the hormonal state of the individual, while the hormonal influence on the responses of the myometrium to non-competitive antispasmodics, under the condition of excess extracellular Ca²⁺, is not clear. The effect of papaverine, a non-competitive antispasmodic, on the guinea pig and rabbit intestinal smooth muscles is known to be antagonized by excess external Ca²⁺. Fleckenstein et al. have reported that the antioxytocin action of papaverine can be neutralized to a great extent by increasing the Ca²⁺ concentration in the bathing medium in the isolated rat myometrium, while Uruno et al. and Uruno et al. have observed in the ovariectomized rat uterus that its antioxytocin action is not antagonized by elevation of the external Ca²⁺. This difference in the antioxytocin action of papaverine in the presence of excess external Ca²⁺ indicates the possibility that sex hormone(s) may affect the cell membrane and/or intracellular organelles to produce changes in Ca exchangeability through the membrane and/or in Ca content of the uterine muscle.

In this paper, the influence of Ca²⁺ on antioxytocin actions of the three non-competitive antispasmodics, papaverine, deoxycholate and 1,1-diphenyl-3-piperidinobutanol hydrochloride (Aspaminol), was investigated in the ovariectomized and the estradiol-treated rat uteri. In addition, Ca exchangeability and Ca content in the ovariectomized rat longitudinal uterine muscle were compared with those in the estradiol-treated one.

METHODS AND MATERIALS

Animals — Virgin female Wistar rats weighing 150 to 250 g were primed by a subcutaneous
injection of estradiol benzoate (1.0 mg/kg) the day before the experiment (estradiol-treated rats), or they were ovariectomized by the dorsal route, under ether anaesthesia, 24 hr after having been primed by the subcutaneous injection of estradiol benzoate (1.0 mg/kg, ovariectomized rats) and allowed to recover for more than a week before the experiment. In some experiments, the ovariectomized rats were treated with estradiol benzoate (1.0 mg/kg) once a day for 2 days (ovariectomized estradiol-treated rats).

Measurement of Contractile Responses — The animal was stunned by a blow on the head and the uterine horns were removed. The horns were then placed in 10 ml organ baths containing Locke–Ringer’s solution, kept at 32° and gassed continuously with air. The Locke–Ringer’s solution used contained NaCl 154 mM, KCl 5.4 mM, CaCl₂ 2.2 mM, MgCl₂ 2.1 mM, NaHCO₃ 5.9 mM and glucose 2.8 mM. Uterine contractions were isotonically recorded by means of a lever loaded with 0.5 g on a smoked drum. Non-competitive antispasmodics, papaverine, deoxycholate and 1,1-diphenyl-3-piperidinobutanol hydrochloride (Aspaminol), were allowed to act for 5 min and in their presence oxytocin was added. When a high calcium concentration was required, CaCl₂ was added from concentrated stock solution to the bathing medium and 1 min later the antispasmodic was applied. Antoxytocin activities were presented as \( pD_2 \) values, which are the negative logarithms of the molar concentrations of the non-competitive antispasmodics necessary to reduce the maximal responses to oxytocin by 50%. A single supramaximal concentration of oxytocin \( (10^{-2} \text{ units/ml}) \) was used to determine the maximal response and this concentration was applied several times to determine reproducibility of responses.

Determination of Tissue Ca Content and \( ^{45}\text{Ca} \)

![Graph showing contractions induced by oxytocin](image)

**FIG. 1. Contractions induced by Oxytocin in the Uterine Horns and Longitudinal Muscles**

Isotonic tension was detected by an isotonic transducer (ME-4012, ME Commercial Co. Ltd., Tokyo) and recorded on a paper recorder at the magnification indicated in the parenthesis. Oxytocin was cumulatively added in the experiments with the ovariectomized rat uterus.
Space—The estradiol-treated or the ovariectomized rat was stunned by a blow on the head, the uterine horns were rapidly removed and placed in tris buffer solution of the following composition,\(^{13}\) NaCl 125 mM, KCl 2.7 mM, MgCl\(_2\) 1.2 mM, CaCl\(_2\) 1.8 mM, tris(hydroxymethyl)aminomethane 23.8 mM (pH 7.4) and glucose 11.0 mM. The uterine horn in the solution, bubbled continuously with 100% \(\text{O}_2\) at room temperature (26°), was freed from fat and loosely bound connective tissue, and inserted with a fine glass rod. One end of the horn was then fixed on the glass rod with cotton thread and a gentle incision was made along the thread. With a help of a pair of forceps, the longitudinal muscle layer was separated from the rest of the horn by tearing it gently in a longitudinal direction under a binocular stereoscopic microscope (JMT, Olympus Co. Ltd., Tokyo) at 20 to 80 times magnification. The longitudinal muscle layer with some circular muscle attached by such procedures showed satisfactory contractile responses to oxytocin (Fig. 1).

For total tissue Ca determination, the longitudinal muscle layer was allowed to equilibrate, kept at 32°, for 60 min in the oxygenated tris buffer. The muscle layer then gently blotted on Whatman 42 filter paper, rinsed in a Ca\(^{2+}\)-free tris solution similar to the incubation solution for 10 sec, blotted again, placed the muscle in a quartz test tube, and dried in an oven at 100° for 12 hr to obtain dry weight of the muscle. Non-displaceable Ca by La was estimated by the lanthanum method of van Breemen et al.\(^{14}\) This method does not allow an estimate of intracellular Ca.\(^{15,16}\) Therefore, La should be used in order to estimate Ca exchangeability or Ca fraction resistant to displacement by La.\(^{17}\) After equilibration with the oxygenated tris buffer, the longitudinal muscle layer was placed in the oxygenated La\(^{3+}\)-tris buffer, in which 2.0 mM LaCl\(_3\) was substituted for 1.8 mM CaCl\(_2\) in the normal tris buffer solution, and incubated for 60 min. The muscle layer was then gently blotted, dried in the oven at 100° for 12 hr and weighed to obtain dry weight. The dry tissue was ashed in a muffle furnace at 500° for 16 hr. The residue was dissolved in 0.1 N HCl and aliquots were taken for the atomic absorption spectrophotometric assay of calcium.

For measurement of \(^{45}\text{Ca}\) tissue space, after equilibration with the oxygenated tris buffer solution, kept at 32°, the longitudinal muscle layer incubated in the labelled tris buffer solution (\(^{45}\text{Ca} ; 0.1 \mu \text{Ci/ml}\) was withdrawn after various times of incubation at 32°. After the desired time interval, the muscle layer was transferred into an organ bath containing the oxygenated Ca\(^{2+}\)-free, La\(^{3+}\)-tris buffer (La\(^{3+}\); 2 mM or 10 mM) and incubated for 60 min at 32°. The tissue was then removed from the La\(^{3+}\)-tris buffer solution, blotted gently with Whatman 42 filter paper, and weighed. In some experiments, dry weight was obtained by the same procedure as the total tissue Ca determination. Total uptake of \(^{45}\text{Ca}\) before lanthanum treatment was measured by rinsing the muscle layer in the La\(^{3+}\)- and isotope-free solution for 0, 15 or 60 sec. The wet or dried tissue was placed in 1 ml Protosol, a tissue solubilizer, and incubated for 4 hr at 50° to solubilize the tissue. Aliquots of this solution were used for the radioassay of exchangeable Ca by liquid scintillation counting. The \(^{45}\text{Ca}\) space was calculated as the ratio between the radioactivity (cpm) of the muscle and of the bathing medium.\(^{13}\)

Sorbitol Space — [H]-sorbitol was used to determine extracellular spaces of uterine longitudinal muscle layers. After equilibration with the oxygenated tris buffer solution at 32° for 60 min, the muscle layer was placed in the oxygenated [H]-sorbitol-tris buffer (0.2 \mu Ci/ml) kept at 32°. After the desired time interval (2, 5, 10, 30, 60 and 120 min), the muscle was rinsed in the [H]-sorbitol-free tris buffer solution for a few seconds, blotted gently with Whatman 42 filter paper and weighed. The weighed muscle was then placed in 1 ml Protosol and incubated for 4 hr at 50° to solubilize the muscle. Aliquots of this solution were used for the radioassay. The spaces occupied by sorbitol were expressed as ml/100 g of the initial weight.

Statistical Analysis — The data were analyzed by means of Student’s \(t\)-test and statistical signifi-
TABLE I. Influence of Extracellular Ca\textsuperscript{2+} on the pD\textsubscript{2}' Values of Deoxycholate and Aspaminol in the Ovariectomized and the Estradiol-treated Rat Uteri

<table>
<thead>
<tr>
<th>Ca\textsuperscript{2+} (mM)</th>
<th>Ovariectomized\textsuperscript{a)}</th>
<th>Estradiol-treated</th>
<th>Deoxycholate</th>
<th>Aspaminol</th>
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<td>Ca\textsuperscript{2+} (mM)</td>
<td>Deoxycholate</td>
<td>Aspaminol</td>
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<td>2.2</td>
<td>3.71±0.05 (12) (a)</td>
<td>4.33±0.07 (6) (d)</td>
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<td></td>
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<td>5.0</td>
<td>3.73±0.07 (12) (b)</td>
<td>3.97±0.05 (6) (e)</td>
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<td></td>
<td>10.0</td>
<td>3.86±0.07 (12) (c)</td>
<td>3.86±0.03 (6) (f)</td>
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Estradiol-treated

<table>
<thead>
<tr>
<th>Ca\textsuperscript{2+} (mM)</th>
<th>Deoxycholate</th>
<th>Aspaminol</th>
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Each value represents the mean ± S.E. of the number of experiments indicated in the parenthesis. Significance of differences at the p < 0.05 level; (a, g), (d, j), (b, h), (c, i), (d, e), (d, f), (j, k) and (j, l).

a) The pD\textsubscript{2}' values in the ovariectomized rat uterus were cited from the data of Uruno et al.\textsuperscript{10} for comparison.

RESULTS

Table I summarizes the influence of extracellular Ca\textsuperscript{2+} on the pD\textsubscript{2}' values of deoxycholate and Aspaminol in the uteri of the ovariectomized and the estradiol-treated rats. In normal Locke-Ringer's solution (Ca\textsuperscript{2+}; 2.2 mM), significant differences were observed between the pD\textsubscript{2}' values of deoxycholate in the ovariectomized and the estradiol-treated rat uterus, i.e. the antioxytocin action of deoxycholate in the estradiol-treated rat uterus was weaker than that in the ovariectomized one. Elevation of the Ca\textsuperscript{2+} concentration in the bathing medium to 5.0 or 10.0 mM did not have significant effect on the pD\textsubscript{2}' values of deoxycholate in both ovariectomized and estrogenized rat uterus, although its antioxytocin action at the higher Ca\textsuperscript{2+} concentrations tended to be strengthen as compared with that at the 2.2 mM Ca\textsuperscript{2+} concentration. Meanwhile, there were significant differences between the pD\textsubscript{2}' values of Aspaminol at the normal and higher Ca\textsuperscript{2+} concentrations in both ovariectomized and estrogenized rat uterus, i.e. the antioxytocin action was significantly antagonized by elevation of the external Ca\textsuperscript{2+} concentration to 5.0 or 10.0 mM in

TABLE II. Influence of Extracellular Ca\textsuperscript{2+} on the pD\textsubscript{2}' Values of Papaverine in the Ovariectomized, the Estradiol-treated and the Ovariectomized Estradiol-treated Rat Uteri

<table>
<thead>
<tr>
<th>Ca\textsuperscript{2+} (mM)</th>
<th>Ovariectomized\textsuperscript{a)}</th>
<th>Estradiol-treated without ovariectomy</th>
<th>Estradiol-treated for 2 days after ovariectomy</th>
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<td></td>
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<td>2.2</td>
<td>4.62±0.06 (8) (a)</td>
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<td></td>
<td></td>
<td>5.0</td>
<td>4.60±0.12 (10) (b)</td>
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<td></td>
<td>10.0</td>
<td>4.45±0.07 (8) (c)</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E. of the number of experiments indicated in the parenthesis. Significance of differences at the p < 0.05 level; (a, g), (b, e), (c, f), (c, h), (d, e), (d, f), (d, g), (g, h) and (j, l).

a) The pD\textsubscript{2}' values in the ovariectomized rat uterus were cited from the data of Uruno et al.\textsuperscript{10} for comparison.
by the excess Ca$_2^+$, while in the estradiol-treated rat uterus the pD$_5$' values at the Ca$_2^+$ concentration of 5.0 or 10.0 mM were significantly lower as compared with the control value at the Ca$_2^+$ concentration of 2.2 mM, i.e. the antioxytocin action of papaverine was reduced by elevation of the external Ca$_2^+$ concentrations. These results suggest that the difference in Ca$_2^+$-papaverine interaction in the ovariectomized and the estrogenized rat uteri may be related to the hormonal state of the animal. In order to investigate the effect of estradiol on the Ca$_2^+$-papaverine interaction, the pD$_5$' values of papaverine were determined in the uterus treated with estradiol (1.0 mg/kg) for 2 days after ovariectomy. In the ovariectomized estradiol-treated rat uterus as well as in the estradiol-treated one, the antioxytocin action was antagonized by the excess external Ca$_2^+$, although the effect of Ca$_2^+$ on the antioxytocin action of papaverine in the ovariectomized estradiol-treated uterus was reduced as compared with that in the estradiol-treated one (Table II).

In order to investigate whether the difference in Ca$_2^+$-papaverine interaction in the ovariectomized and the estrogenized rat uteri is due to the differences in Ca$_2^+$ exchangeability through the cell membrane and/or Ca content between the uteri ovariectomized and treated with estradiol, total tissue Ca content, La-resistant Ca content and Ca exchangeability in the ovariectomized rat uterine longitudinal muscle layers, obtained by removal of endometrium and most circular muscle, were compared with those in the estradiol-treated ones. The La-resistant Ca content was determined by exposing 2.0 mM LaCl$_3$ to the longitudinal muscle for 1 hr. Table III shows the total Ca content and La-resistant Ca content of the ovariectomized and the estradiol-treated rat uterine longitudinal muscles. No significant differences were observed both between the total Ca content and between La-resistant Ca content of both uterine longitudinal muscles.

Fig. 2 shows the total uptake of $^{45}$Ca before lanthanum washing in the ovariectomized and the estradiol-treated rat uterine longitudinal muscles. After different times of incubation in the tris
FIG. 3. $^{45}$Ca Space after Lanthanum Washing for 1 hr in the Ovariectomized and the Estradiol-treated Rat Uterine Longitudinal Muscles

$^{45}$Ca space is expressed in terms of non-displaceable $^{45}$Ca by 2 mM (A) or 10 mM (B) La$^{3+}$. (●), ovariectomized; (○), estradiol-treated. Each point is mean ± S.E. from 6 to 12 experiments. For clarity, the standard error is shown in one direction only.

FIG. 4. Uptake $[^3$H]-Sorbitol by the Ovariectomized and the Estradiol Treated Uterine Longitudinal Muscles

(●) ovariectomized; (○), estradiol-treated. Each point is mean ± S.E. from 3 to 6 experiments. For clarity, the standard error is shown in one direction only.

solution containing $^{45}$Ca, the muscles were rinsed in the $^{45}$Ca-free solution for 0, 15 or 60 sec. The ovariectomized myometrium without rinsing and with rinsing for 15 sec took up more $^{45}$Ca than the estrogenized ones. This relationship was, however, reversed by 60-sec rinsing. These data show that $^{45}$Ca loss during 60 sec into $^{45}$Ca-free solution was larger in the ovariectomized longitudinal muscle than that in the estrogenized one.

$^{45}$Ca spaces after lanthanum washing for 1 hr in the ovariectomized and the estradiol-treated rat uterine longitudinal muscles are illustrated in Fig. 3. The $^{45}$Ca space is expressed in terms of non-displaceable $^{45}$Ca by 2 mM La$^{3+}$ (Fig. 3A) or 10 mM La$^{3+}$ (Fig. 3B). $^{45}$Ca space after 2 mM La$^{3+}$ washing in the ovariectomized longitudinal myometrium reached a steady state within 5 min, while in the estrogenized one the amount of La-resistant $^{45}$Ca fraction was significantly increased with incubation after 5 min (Fig. 3A). The similar results were obtained after 10 mM La$^{3+}$ washing, although the extent of the increase in $^{45}$Ca space throughout the incubation time in the estrogenized myometrium was smaller as com-
pared with the muscle after 2 mM La\(^{3+}\) washing. Strict comparison is, however, impossible since the experiments for Fig. 3A and 3B carried out under different experimental conditions (\(^{45}\)Ca space in Fig. 3A is expressed in terms of ml/dry wt).

Fig. 4 shows the uptake of \(^{3}H\)-sorbitol by the uterine longitudinal muscles ovarieotomized and treated with estradiol. The size of extracellular spaces determined by \(^{3}H\)-sorbitol was found to be larger in the estrogenized myometrium as compared with that in the ovarieotomized one. The uptake of sorbitol reached a steady value within 30 min in the ovarieotomized muscle, while slight increase in the size of the sorbitol spaces after 30 min of incubation with sorbitol was found in the estradiol-treated one.

DISCUSSION

The antioxytocin action of Aspaminol in both ovarieotomized and estradiol-treated rat uterus was significantly antagonized by the excess external Ca\(^{2+}\), while that of deoxycholate in both uterus was little influenced by elevation of the external Ca\(^{2+}\) concentrations (Table I). Thus, no qualitative difference in the responses to deoxycholate and Aspaminol at the normal and higher Ca\(^{2+}\) concentrations in the estrogenized uterus was seen as compared with those obtained from the ovarieotomized one. However, the pD\(_{2}\) values of deoxycholate at the normal and higher Ca\(^{2+}\) concentrations in the estrogenized rat uterus were significantly lower than those in the ovarieotomized one and the value of Aspaminol at the normal Ca\(^{2+}\) concentration in the estradiol-treated rat uterus was also lower than that in the ovarieotomized one. These results suggest that the qualitative effects of deoxycholate and Aspaminol may not depend upon the hormonal state of the rat.

In the ovarieotomized rat uterus, Uruno et al.\(^{9}\) and Uruno et al.\(^{10}\) have observed that the antioxytocin action of papavereine is not antagonized by increasing the external Ca\(^{2+}\) concentrations. In this present study with the estradiol-treated rat uterus, the antioxytocin action of papavereine was significantly antagonized by the excess external Ca\(^{2+}\) (Table II). The latter is in agreement with the observation of Fleckenstein et al.\(^{8}\) who showed that the antioxytocin action of papavereine can be antagonized by increasing the external Ca\(^{2+}\) concentration in the isolated rat myometrium. Our results mentioned above support the proposals that there are different mechanisms of action of these non-competitive antispasmodics.\(^{10,18,19}\)

The interesting feature of the effect of papavereine is that its antioxytocin action was antagonized by the excess external Ca\(^{2+}\) in the estrogenized rat uterus, but not in the ovarieotomized one. This difference in the effects of external Ca\(^{2+}\) on the antioxytocin action of papavereine in the ovarieotomized and the estradiol-treated rat uteri is considered to be due to the difference in the hormonal environment of the myometrium. Comparison of the effect of the excess external Ca\(^{2+}\) on the pD\(_{2}\) values of papavereine in the estradiol-treated rat uterus with that in the ovarieotomized one clearly shows that, even in the ovarieotomized estradiol-treated rat uterus as well as in the estradiol-treated one, the antioxytocin action of papavereine was antagonized by the excess external Ca\(^{2+}\), although its values in the estradiol-treated uterus were higher than those in the ovarieotomized estradiol-treated one (Table II). These results indicate that antagonism of papavereine against exogenous Ca\(^{2+}\) in the estradiol-treated rat uterus may be concerned with the estradiol-dominated state of the rat.

The difference in the effect of Ca\(^{2+}\) on the antioxytocin action of papavereine in the ovarieotomized and the estradiol-treated rat uteri might be related to changes in Ca\(^{2+}\) exchangeability and/or Ca content in both uteri. In order to investigate Ca distribution and movements in rat myometrium, uterine longitudinal muscle layers were obtained by removal of endometrium and most circular muscle. The muscle layers obtained showed satisfactory contractile responses to oxytocin (Fig. 1). In addition, the uptake of sorbitol reached a steady state within 30 min in the ovarieotomized longitudinal muscle (Fig. 4). Thus, the longitudinal muscles obtained appear to
be satisfactory for our experiments. The increase in sorbitol spaces observed in the estradiol-treated longitudinal muscle (Fig. 4) may be due to the increased membrane permeability rather than damage of cell membrane.

There were no significant differences either in the total tissue Ca content or the non-displaceable Ca by La between the ovariectomized and the estradiol-treated rat uterine longitudinal muscle layers (Table III). These results suggest that the difference in the effect of Ca$^{2+}$ on the antioxytocin action of papaverine in the ovariectomized and the estrogenized myometria could not be explained by the differences in the total tissue Ca content or La-insensitive Ca fraction. The possibility cannot be, however, eliminated that a hormonal influence on the myometrial calcium content may be undetected since its content is considered to be so small relative to the total amount.

The loss of $^{45}$Ca into $^{45}$Ca-free solution within 60 sec in the estradiol-treated uterine longitudinal muscle was smaller as compared to that in the ovariectomized muscle (Fig. 2), although the size of sorbitol spaces after incubation of more than 10 min with sorbitol in the estrogenized myometrium was larger than that in the ovariectomized one. These results suggest that the ovariectomized longitudinal muscle may have more $^{45}$Ca exchanged with Ca and bound loosely in extracellular space and/or at superficial membrane binding sites, since the early rapid efflux probably represents $^{45}$Ca in the extracellular tissue space and/or extracellular $^{45}$Ca in loose association with the cell membrane. This increased exchange of $^{45}$Ca in the ovariectomized myometrium seems not to play an important role in antagonism of Ca$^{2+}$ against the antioxytocin action of papaverine, considering our result that its antioxytocin action was not antagonized by increasing extracellular Ca$^{2+}$ in the ovariectomized myometrium. The Ca fractions exchanged with $^{45}$Ca in the extracellular and/or superficial membrane binding sites, however, appear to be important for antagonism of extracellular Ca$^{2+}$ against the antioxytocin action of Aspaminol. Takayanagi et al. $^{6}$ have reported that Aspaminol inhibits the calcium uptake by the microsomal fraction of rabbit taenia coli, and suggested its action may be on the surface of intestinal smooth muscle.

A hormonal influence on the contractility$^{20-22}$ and $^{45}$Ca uptake$^{23}$ of the myometrium has been reported. In our present paper, we investigated $^{45}$Ca uptake after lanthanum washing (La$^{3+}$: 2 mM or 10 mM) for 1 hr in the ovariectomized and estrogenized uterine longitudinal muscles. More increase in the La$^{3+}$-resistant $^{45}$Ca space was observed in the estrogenized muscle (Fig. 3). This indicates that Ca exchangeability in the estradiol-treated longitudinal muscle is much greater than that in the ovariectomized one. Since La$^{3+}$ displaces extracellular bound Ca, $^{14,24}$ the increased $^{45}$Ca space may be mainly derived from cellular $^{45}$Ca. When the estrogenized myometrium taken up $^{45}$Ca was incubated with Ca$^{2+}$-free tris solution containing 10 mM La$^{3+}$ for 1 hr, the $^{45}$Ca space was significantly decreased as compared to the 2 mM-lantnanum treatment (Fig.

### TABLE III. Total and La$^{3+}$-resistant Ca Content of the Ovariectomized and the Estradiol-treated Rat Uterine Longitudinal Muscles

<table>
<thead>
<tr>
<th>Solution</th>
<th>Ovariectomized</th>
<th>Estradiol-treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal tris$^a$</td>
<td>6.01 ± 0.49 (8)</td>
<td>6.61 ± 0.19 (8)</td>
</tr>
<tr>
<td>La$^{3+}$-tris$^b$</td>
<td>1.67 ± 0.21 (8)</td>
<td>1.57 ± 0.25 (8)</td>
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</table>

Each value represents the mean ± S.E. of the number of experiments indicated in the parenthesis. Data are expressed in terms of μmol Ca per g dry tissue weight.

$^a$ The medium contains 125.0 mM NaCl, 2.7 mM KCl, 1.8 mM CaCl$_2$, 1.2 mM MgCl$_2$, 11.0 mM glucose and 23.8 mM tris (hydroxymethyl)aminomethane (pH 7.4).

$^b$ 2.0 mM LaCl$_3$ was substituted for 1.8 mM CaCl$_2$ in the normal tris solution.
3. This results suggest that 2 mM La\textsuperscript{3+} could not displace all extracellular bound \textsuperscript{45}Ca, or that \textsuperscript{45}Ca located at less superficial or more sequestered sites may be displaced by La\textsuperscript{3+} since it has been reported that La\textsuperscript{3+} enters cells and become associated with mitochondria and/or endoplasmic reticulum.\textsuperscript{15} Kato et al.\textsuperscript{25} investigated the effect of Ca\textsuperscript{2+} transported into the uterine muscular cell through the cell membrane on actomyosin-ATPase activity of the uterus during pregnancy and delivery, and demonstrated that Ca\textsuperscript{2+} cannot enter the muscular cell during pregnancy, but can do so during delivery. They suggested this permeability of Ca\textsuperscript{2+} through the cell membrane is controlled by estrogens. On the basis of our results and their observations, it is concluded that cellular Ca exchangeability in the estradiol-dominated rat uterus is much greater than that in the ovarioctomized one and that this increased Ca exchangeability may play an important role in the antagonism of papaverine against excess extracellular Ca\textsuperscript{2+} and the lower pD\textsubscript{2} values in the estrogenized myometrium. However, our results are as yet insufficient to provide an explanation to the mechanism by which estrogens produce the difference in the effect of external Ca\textsuperscript{2+} on the antioxytocin action of papaverine in the ovarioctomized and the estradiol-treated rat uteri. Carsten\textsuperscript{26} has reported that oxytocin inhibits ATP-dependent calcium binding in sarcoplasmic reticulum preparations from pregnant and non-pregnant bovine uterus and that the inhibition of calcium binding by oxytocin is greater in the third trimester of pregnancy than in the non-pregnant bovine uterus. Therefore, one cannot eliminate the possibility that the effect of hormonal state in the animal on sarcoplasmic reticulum and/or mitochondria may produce the antagonism of papaverine against extracellular Ca\textsuperscript{2+}. In addition, the possibility cannot be ruled out that there is some effect of estradiol-treatment on oxytocin receptors, although the similar difference in the effect of excess exogenous Ca\textsuperscript{2+} on the non-competitive antiacetylcholine action of papaverine in the ovarioctomized and the estradiol-treated rat uteri has been observed (unpublished observation). Both antioxytocin action of papaverine and effect of the external Ca\textsuperscript{2+} on the antioxytocin action of papaverine in the ovarioctomized estradiol-treated rat uterus were weaker than those in the estradiol-treated one (Table II). These may result from independence of the endogenous progesterone supply by ovarioctomy, which suppress intrinsic excitation, conduction and pharmacological reactivity in uterine muscle.\textsuperscript{27}

Our results also suggest the possibility that papaverine may develop its antioxytocin action intracellularly and/or by preventing transmembrane Ca\textsuperscript{2+} fluxes in the rat myometrium, while the site of action of Aspaminol, as suggested in the intestinal smooth muscle by Takayanagi et al.,\textsuperscript{6} may be on the surface of the cell membrane. The antioxytocin action of deoxycholate clearly differs from that of papaverine or Aspaminol, although its mechanism of action is not clear. Since the antioxytocin action of deoxycholate at the concentrations used in the present study is reversible in the rat myometrium, irreversible tissue damage by the surface active action of deoxycholate could not be ruled out. Although it has been reported that papaverine and the bile salt increase cyclic AMP level in the ovarioctomized rat uterus,\textsuperscript{9,19} the relationship between the increase in cyclic AMP level and the antagonism of papaverine against excess exogenous Ca\textsuperscript{2+} is at present unknown. Ca movements in the presence of agonists and/or antagonists in the ovarioctomized and estrogenized rat myometria are now under investigation.

REFERENCES
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