Hypothyroid state reduces calcium channel function in 18-day pregnant rat uterus

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Hypothyroidism significantly reduced the mean amplitude and increased the mean frequency of spontaneous rhythmic contractions in 18-day pregnant rat uterus. Nifedipine (10−12-10−9 M) and diltiazem (10−10-10−6 M) caused concentration related inhibition of the myogenic responses of the uterine strips obtained from both pregnant and hypothyroid state. However, nifedipine was less potent (IC50:2.11 × 10−11 M) in inhibiting the uterine spontaneous contractions in hypothyroid than in pregnant rat uterus (IC50:5.37 × 10−12 M). A similar decrease in the sensitivity to nifedipine and diltiazem for reversal of K⁺ (100 mM)-induced tonic contraction and K⁺-stimulated 45Ca²⁺ influx was observed with these calcium channel antagonists in uterus obtained from hypothyroid pregnant rats compared to the controls. Nifedipine-sensitive influx of 45Ca²⁺-stimulated either by K⁺ (100 mM) or by Bay K8644 (1,4-dihydro-2,6-methyl-5-nitro-4-[2'-tri-fluoromethyl]phenyl]-3-pyridine carboxylic acid methyl ester) (10−9 M) was significantly less in uterine strips from hypothyroid rats compared to controls. The results suggest that the inhibition of uterine rhythmic contractions may be attributable to a reduction in rat myometrial Ca²⁺ channel function in the hypothyroid state.

Key words: Calcium, Hypothyroidism, Pregnancy, Uterus

Reproductive dysfunctions, ranging from abnormal sexual development to infertility and irregularities in reproductive cycle, have long been associated with thyroid disorders1. Hypothyroidism has also been reported to influence uterine morphology. For instance, in rats made hypothyroid with methimazole, there is a reduction in absolute volume of endometrium and a decrease in muscle layer2. Although there is evidence for the presence of thyroid hormone receptors in uterine nuclei and of a very significant influence of thyroid hormones on the contractility of myometrium3-5, there is little information on the influence of thyroid status on the cellular basis of altered contractility, especially with respect to Ca²⁺ handling by the tissues. There are a number of reports, however, on the effect of thyroid status on calcium channel function in the cardiovascular system. For instance, ventricular myocytes obtained from hypothyroid guinea pigs showed a reduced peak Ca²⁺ channel current6. It has been shown that hypothyroidism induces a moderately lower (reduced by about 23%) myocardial density of 1,4-dihydropyridine receptors in rats7. Hypothyroidism, on the other hand, increased the calcium channel function in chick ventricular myocytes and human atrial myocytes. Chick ventricular cells grown in triiodothyronine (T3) showed a marked increase in dihydropyridine binding and an augmented trans-sarcolemmal Ca²⁺ influx8. The increased calcium channel function was also evident in patients with latent hypothyroidism (a condition in which there is a suppressed thyroid hormones) wherein there was an increased expression and activity of L-type Ca²⁺ channels9.

Calcium flux is an essential component for excitation-contraction coupling in uterine smooth muscle10-13. Pregnancy, or more specifically, the hormonal state induced by pregnancy, can influence resting membrane potential and ion channel composition in the myometrium14-19. Ion channels in myometrium and other gene products such as gap junction proteins20 and oxytocin receptors21 may be important constituents that move the myometrium from a state of relative quiescence to a rhythmically contracting organ at the time of parturition. Voltage dependent L-type calcium channels (VDCC) have

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been identified in uterine myometrium by electrophysiologic, pharmacologic and molecular studies. They are responsible for the majority of the observed calcium current in term human myometrium. These channels are subject to hormonal regulation as evident from an increase in channel density with corresponding increase in \( ^{45} \text{Ca}^{2+} \) influx in the myometrium of estrogen-treated rat uterus. In vitro application of \( \beta \)-estradiol has been reported to inhibit calcium channel currents in pregnant rat myometrial cells. In virgin rat uterus, hypothyroidism reduces the spontaneous rhythmic contractions and the influx of \( \text{Ca}^{2+} \) through voltage-dependent L-type \( \text{Ca}^{2+} \) channels, as well as decrease the sensitivity of the smooth muscle to L-type \( \text{Ca}^{2+} \) channel blockers such as nifedipine and diltiazem. Further, it is not yet known that such influence of hypothyroidism does exist in 18-day pregnant uterus or not. In view of the lack of information concerning the role of thyroid hormones in affecting \( \text{Ca}^{2+} \) channel function in the pregnant myometrium, the aim of the present study is to examine the influence of methimazole-induced hypothyroidism on the depolarization induced \( ^{45} \text{Ca}^{2+} \) influx and on the sensitivity of spontaneously contracting rat uterus to calcium channel blockers such as nifedipine and diltiazem. There is also no information on the influence of hypothyroidism on spontaneous rhythmic contractions of pregnant rat uterus. It was therefore thought interesting to examine the link between calcium channel function and mechanical responses as influenced by the thyroid status.

**Materials and Methods**

Adult virgin female Sprague-Dawley rats (180-200 g), obtained from the Central Drug Research Institute, Lucknow, were used as per the Institutional Policy on Animal Use. Two to three rats were kept to mate with one matured male rat. Vaginal smear was examined under dissecting microscope for presence of sperm for two consecutive days. The day of mating was detected by the presence of sperm in the vaginal smear and denoted as day 0. 0-day pregnant rats in the test group were treated with methimazole (10 mg kg\(^{-1} \) day\(^{-1} \), ip) for two weeks as per method of Swann. Pregnant control rats received a comparable volume of normal saline ip. The thyroid status in control and methimazole-treated rats was evaluated by measuring plasma thyroxine (T\(_4\)) and triiodothyronine (T\(_3\)) using a radioimmunoassay.

The neonates were removed carefully after incising longitudinally the uterine horn of the animal under ether anaesthesia. Then horns were isolated and placed in cold oxygenated Ringer-Locke solution having (mM): NaCl, 136.9; KCl, 5.6; NaHCO\(_3\), 11.9; CaCl\(_2\), 2H\(_2\)O, 2.2 and glucose, 5.6. The longitudinal strips, approximately 3x10 mm from each mid-horn region, were dissected out and suspended in an organ bath containing 20 ml Ringer-Locke modified solution, bubbled continuously with O\(_2\) at 37\(^\circ\)C±0.5\(^\circ\)C under a resting tension of 1g and equilibrated for 1 hr. Isometric contractions were recorded by a force transducer connected to an ink-writing oscillograph (Recorder and Medicare, India).

\( ^{45} \text{Ca}^{2+} \) influx measurement—The net increase in the unidirectional entry of \( \text{Ca}^{2+} \) into the uterine smooth muscle was estimated by measuring the increase in \( ^{45} \text{Ca}^{2+} \) content of uterine strips obtained from pregnant and hypothyroid rats as per the method of Batra. In brief, the uterine strips weighing about 6-8 mg were equilibrated for 45 min in physiological Na-HEPES solution comprising (mM): NaCl, 135; KCl, 4.6; MgCl\(_2\), 1.2; CaCl\(_2\), 1.5; glucose, 11 and HEPES, 10; pH 7.4, maintained at 37\(^\circ\)C and bubbled with O\(_2\). The tissues were incubated for 15 min with different concentrations of calcium channel blockers such as nifedipine (10\(^{-11}\)-10\(^{-8}\) M) and diltiazem (10\(^{-9}\)-10\(^{-6}\) M) before the strips were equilibrated for an additional 2 min with Na-HEPES solution containing \( ^{45} \text{Ca}^{2+} \) (1-1.5 \(\mu\)Ci/ml) to allow the exchange of extracellular \( \text{Ca}^{2+} \) with the tracer. For stimulation of \( ^{45} \text{Ca}^{2+} \) influx into the strips, the tissues were exposed for 2 min to a \( ^{45} \text{Ca}^{2+} \)-containing K\(^{-}\)-depolarising solution in which NaCl was replaced by 100 mM KCl. Unstimulated influx was measured in the tissues incubated in Na-HEPES solution. Uterine strips were then washed for 45 min in 5 ml ice-cold Ca\(^{2+}\)-free EGTA-HEPES solution with vigorous bubbling with O\(_2\) to remove the loosely bound extracellular tracer. After washing with EGTA solution, the tissues were lightly blotted with filter paper, placed in the scintillation vials and weighed. The tissues were digested using the method of Pfeffer et al. The radioactivity of the tissues was measured using a liquid scintillation counter (LKB, USA).

**Drugs**—Nifedipine and methimazole were purchased from Sigma (USA). Diltiazem was a gift from Marion Laboratory (USA). 1’,4-dihydro-2,6-dimethyl-5-nitro-4-[2’-(trifluoro-methyl)-3-pyridine carboxyl]ic acid methyl ester (Bay K8644) was a gift from Bayer (Germany).
Statistical analysis—Results are given as means ± S.E. Student’s t-test was employed to test for significance at the level of P<0.05. Inhibitory concentration (IC₅₀) values were calculated by regression analysis.40

Results

Effects of methimazole on plasma T₃ and T₄ levels—A significant (P< 0.01) decrease in plasma T₃ level (0.54±0.02 ng/ml; n=24), as compared to that of saline-treated euthyroid pregnant controls (1.45±0.06 ng/ml; n=24) was observed in methimazole treated pregnant rats. Similarly, a significant reduction in T₄ level (28.5±1.5 ng/ml; n=24) occurred in the methimazole treated pregnant group compared to the saline-treated pregnant controls (62.0±2.0 ng/ml; n=24).

Effect of thyroid status on the spontaneous rhythmic contractions of the uterus—The influence of thyroid status on spontaneous rhythmic contractions of pregnant rat uterus is shown in Fig. 1. The mean amplitude of rhythmic contractions in pregnant rat uterus was 5.1±0.2 g (n=23) which was significantly (P<0.05) greater than that for the hypothyroid status (3.06±0.12 g; n=23). Similarly, a increase in the mean frequency of spontaneous contractions (1.48±0.02/min; n=23) was noted in the hypothyroid rat uterus compared to the pregnant controls (0.7±0.02/min; n=23).

Effect of thyroid status on the sensitivity of rat uterus to nifedipine and diltiazem—The inhibitory concentration–response curves for nifedipine and diltiazem with respect to amplitude and rate are presented in Figs 2 and 3, respectively. The mean amplitude of control rhythmic contraction in uterus from pregnant and pregnant hypothyroid rats was 5.1±0.6 g (n=9) and 3.06±0.5 g (n=6), respectively. Nifedipine (10⁻¹²-10⁻⁹ M), added cumulatively at intervals of 1 log unit produced a concentration-related reduction in the amplitude of rhythmic contractions of uterine muscle taken from pregnant and pregnant hypothyroid rats. Rhythmic contractions were abolished by nifedipine (10⁻¹⁰ M ) in pregnant

Fig. 1—Influence of hypothyroid state on the spontaneous rhythmic contractions of pregnant rat uterus and the effect of nifedipine (0.1 nM) on these contractions.

Fig. 2—Effect of thyroid status on the sensitivity of the amplitude of spontaneous rhythmic contractions of pregnant rat uterus to nifedipine and diltiazem (A) and (B) depicts the concentration-related inhibition of the amplitude by nifedipine (10⁻¹²-10⁻⁹ M) and diltiazem (10⁻⁶-10⁻⁶ M), respectively added cumulatively at an interval of one log unit.
uterus whereas about a log unit higher concentration of nifedipine ($10^{-9} M$) was required to abolish the rhythmic concentrations in the uterus obtained from pregnant hypothyroid rats. The reduced potency of nifedipine in inhibiting the spontaneous rhythmic contractions of uterine strips obtained from pregnant hypothyroid rats is evident from the increase in IC$_{50}$ value compared to the pregnant controls as shown in Table 1. In the diltiazem group, the amplitudes of spontaneous rhythmic contractions were $5.67\pm0.2$ g (n=6) and $3.3\pm0.1$ g (n=9) in pregnant and hypothyroid pregnant rat uterus, respectively. As observed with nifedipine, a marked decrease in the sensitivity of the myogenic contractions to the diltiazem was also evident in the pregnant hypothyroid rat uterus. The spontaneous contractions were abolished at $10^{-7}$ and $10^{-6}$ M, respectively, in pregnant and hypothyroid pregnant conditions. The IC$_{50}$ of diltiazem to inhibit the amplitude of spontaneous contractions presented in Table 1.

**Influence of the thyroid status on the reversal of K$^+$-induced tonic contractions by nifedipine and diltiazem**—In isolated rat uterus, K$^+$ (100 mM) depolarising solution induced a biphasic contractile response consisting of a fast phasic component followed by a slow sustained contraction which stabilized in about 5 min. The reversal of tonic contraction by Ca$^{2+}$ channel blockers was evaluated to determine the sensitivity of the Ca$^{2+}$ channel ligands in pregnant and pregnant hypothyroid rat uterus. The mean amplitude of tonic component of K$^+$-induced contraction was $4.16\pm0.14$ g (n=12) and $2.39\pm0.05$ g

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**Fig. 3**—Effect of thyroid status on the sensitivity of the frequency of spontaneous rhythmic contractions of pregnant rat uterus to nifedipine and diltiazem (A) and (B) depicts the concentration-related inhibition of the amplitude by nifedipine ($10^{-12}$-$10^{-9}$ M) and diltiazem ($10^{-10}$-$10^{-6}$ M), respectively added cumulatively at an interval of one log unit.

**Fig. 4**—Effect of thyroid status on the concentration-related inhibition of the tonic component of K$^+$ (100 mM)-contracted isolated pregnant rat uterus by nifedipine (A) and diltiazem (B).
in pregnant and pregnant hypothyroid rat uterus, respectively. Nifedipine \((10^{-12}-10^{-9} M)\), added cumulatively, caused a concentration-related relaxation of K\(^+\)-contracted uterine smooth muscle taken from both pregnant and hypothyroid rats (Fig. 4). Complete reversal of the tonic contraction occurred at \(10^{-9} M\) of nifedipine for both. The tissues obtained from hypothyroid rats, however, had a decreased sensitivity to nifedipine which is evident from the increase in its IC\(_{50}\) value compared to controls (Table 1). In the diltiazem group, the absolute tension of K\(^+\)-induced control tonic contractions in pregnant and hypothyroid tissues were 4.2±0.2 g (n=6) and 3.0±0.3 g (n=8), respectively. The concentrations of diltiazem required to completely reverse the tonic contractions were \(10^{-5}\) and \(10^{-4}\) M, in pregnant and hypothyroid rat uterus, respectively. A marked decrease in the sensitivity to relaxation by diltiazem of K\(^+\)-induced tonic contractions was also observed in the uterine smooth muscle taken from hypothyroid rats with a resultant increase in its IC\(_{50}\) value compared to pregnant control (Table 1).

**Effect of thyroid status on the sensitivity of K\(^+\)-stimulated \(^{45}\)Ca-influx to nifedipine and diltiazem**—The net influx of \(^{45}\)Ca-stimulated by 100 mM K\(^+\) was 0.103±0.01 and 0.189±0.04 µmol/g tissue (n=20) in uterine strips obtained from pregnant and hypothyroid rats, respectively. Nifedipine \((10^{-11}-10^{-8} M)\) and diltiazem \((10^{-9}-10^{-6} M)\) inhibited the K\(^+\) (100 mM)-stimulated net \(^{45}\)Ca-influx in a concentration-related manner (Fig. 5). The concentration-response curves of nifedipine and diltiazem were shifted to the right in hypothyroid pregnant rat uterus as compared to the controls. The IC\(_{50}\) values of nifedipine are presented in Table 1. Similarly, the IC\(_{50}\) of diltiazem for inhibiting \(^{45}\)Ca-influx into uterine strips from hypothyroid rats was significantly greater than in the controls (Table 1).

**Effect of thyroid status on nifedipine-sensitive \(^{45}\)Ca-influx in uterine strips stimulated with high K\(^+\) and Bay K 8644**—The influence of thyroid status on K\(^+\)- and Bay K 8644-stimulated \(^{45}\)Ca-influx in pregnant rat uterine strips is shown in Fig. 6. The unstimulated influx of \(^{45}\)Ca which was not sensitive to block by nifedipine \((10^{-9} M)\), was significantly

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**Table 1**—Effect of nifedipine and diltiazem on the amplitude of rhythmic contractions, K\(^+\)-induced tonic contraction and K\(^+\)-stimulated net \(^{45}\)Ca\(^{2+}\)-influx in pregnant (preg.) and hypothyroid pregnant (Hypo-preg.) rat uterus

<table>
<thead>
<tr>
<th>Thyroid state</th>
<th>Nifedipine (IC(_{50}))</th>
<th>Diltiazem (IC(_{50}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Rhythmic contraction</td>
<td></td>
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</tr>
<tr>
<td>Preg.</td>
<td>3.09×10^{-12} M; (n=9)</td>
<td>5.37×10^{-10} M; (n=6)</td>
</tr>
<tr>
<td>(Amplitude)</td>
<td>C.L.2.32×10^{-12}-4.25×10^{-12} M</td>
<td>C.L.2.49×10^{-10}-1.16×10^{-9} M</td>
</tr>
<tr>
<td>(Rate)</td>
<td>8.13×10^{-12} M; (n=9)</td>
<td>2.19×10^{-9} M; (n=6)</td>
</tr>
<tr>
<td></td>
<td>C.L.5.37×10^{-12}-1.23×10^{-11} M</td>
<td>C.L.1.63×10^{-9}-2.94×10^{-9} M</td>
</tr>
<tr>
<td>Hypo-preg.</td>
<td>2.11×10^{-11} M; (n=9)</td>
<td>3.72×10^{-9} M; (n=6)</td>
</tr>
<tr>
<td>(Amplitude)</td>
<td>C.L.1.4×10^{-11}-3.2×10^{-11} M</td>
<td>C.L.2.6×10^{-9}-5.3×10^{-9} M</td>
</tr>
<tr>
<td>(Rate)</td>
<td>8.13×10^{-11} M; (n=9)</td>
<td>1.41×10^{-7} M; (n=6)</td>
</tr>
<tr>
<td></td>
<td>C.L.7.22×10^{-11}-9.15×10^{-11} M</td>
<td>C.L.0.79×10^{-7}-2.5×10^{-7} M</td>
</tr>
<tr>
<td>2. K(^+)-induced tonic contraction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preg.</td>
<td>4.37×10^{-12} M; (n=8)</td>
<td>6.31×10^{-8} M; (n=6)</td>
</tr>
<tr>
<td></td>
<td>C.L.2.72×10^{-12}-7×10^{-12} M</td>
<td>C.L.4.97×10^{-12}-8×10^{-12} M</td>
</tr>
<tr>
<td>Hypo-preg</td>
<td>1.91×10^{-11} M; (n=8)</td>
<td>1.9×10^{-7} M; (n=8)</td>
</tr>
<tr>
<td></td>
<td>C.L.1×10^{-12}-3.05×10^{-11} M</td>
<td>C.L.1.02×10^{-7}-3.79×10^{-7} M</td>
</tr>
<tr>
<td>3. K(^+)-stimulated net (^{45})Ca(^{2+})-influx</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preg.</td>
<td>1.95×10^{-11} M; (n=9)</td>
<td>1.7×10^{-8} M; (n=8)</td>
</tr>
<tr>
<td></td>
<td>C.L.1.29×10^{-11}-2.93×10^{-11} M</td>
<td>C.L.0.6×10^{-8}-5×10^{-8} M</td>
</tr>
<tr>
<td>Hypo-preg</td>
<td>1.29×10^{-10} M; (n=9)</td>
<td>2.26×10^{-7} M; (n=8)</td>
</tr>
<tr>
<td></td>
<td>C.L.0.6×10^{-10}-2.78×10^{-10} M</td>
<td>C.L.1.11×10^{-7}-4.6×10^{-7} M</td>
</tr>
</tbody>
</table>

C.L. 95% confidence limits; n= Number of observations.
(P<0.05) lower in controls (0.245±0.04 µmol/g tissue; n=12) than in the hypothyroid pregnant rat uterus (0.113±0.01 µmol/g tissue, n=12). A 2-min stimulation with K⁺ (100 mM) markedly increased the ⁴⁵Ca⁻⁻influx into the uterine strips obtained from both pregnant and hypothyroid rats. In terms of absolute values, the ⁴⁵Ca⁻⁻influx in response to K⁺-depolarising solution was greater in the pregnant than pregnant hypothyroid ones. Similarly, Bay K 8644-stimulated ⁴⁵Ca⁻⁻influx in pregnant uterine strips was significantly greater as compared to the tissues obtained from pregnant hypothyroid rats. Pretreatment of these tissues for 15 min with nifedipine (10⁻⁹ M) blocked the ⁴⁵Ca⁻⁻influx stimulated by either K⁺ (100 mM) or Bay K 8644 (10⁻⁸ M) in tissues taken from both pregnant and pregnant hypothyroid rats.

**Discussion**

The important observations of the present study are that (i) the mean amplitude of spontaneous rhythmic contractions is reduced and mean frequency is increased, (ii) the potency of L-type Ca²⁺-channel blockers, nifedipine and diltiazem is reduced, (iii) ⁴⁵Ca⁻⁻influx stimulated by K⁺ depolarisation and Bay K 8644 is significantly increased in uterine tissues obtained from pregnant rats than from the pregnant hypothyroid ones. Hypothyroidism in response to methimazole treatment was confirmed by significant decrease in circulatory T₃ and T₄ levels. The reduced Ca²⁺ channel function may have been responsible for a reduction in the amplitude of rhythmic contractions in hypothyroid uterus. It is well established that both rhythmic contractions and contractions caused by K⁺ depolarisation in uterine smooth muscle are critically dependent on extracellular Ca²⁺ (ref. 13) and L-type Ca²⁺ channel blockers are potent inhibitors of these contractions. Hypothyroidism has been reported to increase the opening of ATP-sensitive K⁺-channels to decrease the expression of voltage-dependent K⁺-channels K⁺ (Kᵥ)-channel mRNAs in rat myocardium and to reduce the potency of the L-type Ca²⁺-channel antagonists in non-pregnant rat uterus. It is possible that the alteration in the mechanical responses of pregnant uterus in hypothyroidism results from a change in both Ca²⁺ and K⁺ channel function. But it is
not known that whether hypothyroidism does influence the L-type Ca$^{2+}$-channel function of pregnant uterine smooth muscles which has a different hormonal influence as compared to non-pregnant one. With a view to achieve this the present studies were restricted to the Ca$^{2+}$-channels function in pregnant uterine smooth muscles as influenced by hypothyroidism.

Nifedipine and diltiazem, the prototype Ca$^{2+}$-channel blockers, caused concentration-related inhibition of spontaneous rhythmic contractions in the uterus taken from both pregnant and pregnant hypothyroid rats. Nifedipine was more potent than diltiazem to inhibit the rhythmic contractions. A rightward shift in the concentration-response curves of nifedipine and diltiazem in hypothyroid tissue indicates that Ca$^{2+}$-channel blockers are less potent to inhibit the uterine contractions in presence of thyroid deficiency. It is known that the membrane potential is a critical determinant of the potency of Ca$^{2+}$-channel blockers to block the L-type Ca$^{2+}$-channels. In order to rule out a possible influence of membrane potential affecting the potency of nifedipine and diltiazem, some experiments were conducted in high-K$^+$ solution that caused a biphasic contraction of the uterus taken from both pregnant and pregnant hypothyroid rats. Nifedipine and diltiazem were less potent to reverse the tonic component of K$^+$ contraction in uterine strips obtained from pregnant hypothyroid rats than those from pregnant ones. The observations from the contraction studies were further confirmed by the findings that concentration-response curves of nifedipine and diltiazem for blocking K$^+$-stimulated $^{45}$Ca$^{2+}$ influx in uterine strips obtained from hypothyroid rats were shifted rightward as compared to pregnant controls. These observations suggest that hypothyroidism reduces the affinity of L-type Ca$^{2+}$-channel blockers. Still there are no reports regarding the regulation of Ca$^{2+}$-channel function in myometrium by thyroid hormones at 18$^{th}$ day pregnancy except some report on only pregnant myometrium.

Consistent with the reduced sensitivity of L-type calcium channels to nifedipine and diltiazem, hypothyroidism markedly inhibited dihydropyridine-sensitive $^{45}$Ca$^{2+}$ influx, stimulated by either high K$^+$ or the Ca$^{2+}$ channel agonist, Bay K 8644 in uterine strips taken from pregnant hypothyroid rat when compared with the pregnant controls. Although there is no information on the effect, there are several reports on the influence of thyroid status on the L-type Ca$^{2+}$-channel function in the myocardium. For instance, hypothyroidism was reported to inhibit the peak L-type Ca$^{2+}$ channel current in guinea-pig ventricular myocytes. Further, in a ligand binding study, hypothyroidism was shown to lower the density of myocardial 1,4-dihydropyridine receptors in rats. Hypothyroidism on the other hand, has been reported to increase cardiac Ca$^{2+}$ channel function. In view of the rat uterus, it is possible that thyroid hormones regulate uterine Ca$^{2+}$ channels in a manner similar to their influence on cardiac Ca$^{2+}$ channels.

Gestational regulation of uterine L-type calcium channels and their role in parturition are not yet completely understood. The functional VDCC is a multisubunit complex consisting of the $\alpha_1$ pore-forming subunit and $\beta$, $\gamma$, and $\omega_2$/$\sigma$ regulatory subunits. Known drug binding sites, including the dihydropyridine (DHP) binding site, are located on the $\alpha_1$ subunit and have been used to help characterize the channel. The $\alpha_1$ subunit from rat uterine myometrium, a product of the CaCh2 gene, has several isoforms resulting from alternative splicing and is DHP sensitive. There is evidence that the L-type calcium channel subunits are regulated during gestation in the rat. Mershon et al. found a 4-fold increase in the number of DHP binding sites, measured by saturation binding analysis, by 14 days of gestation in the rat, a time of supposed uterine quiescence, which was maintained through parturition. There was no further rise immediately prior to or during labor. There was no change in affinity ($K_d$) throughout gestation. Mershon et al. also studied transcript expression of the $\alpha_1$ subunit and showed a gradual rise in mRNA beginning at 7 days (0.3) of gestation with a marked decrease and isoform shift at parturition. Similarly, Tezuka et al. showed a gradual increase in the $\alpha_1$ subunit through gestation, a decrease during labor, but a sharp increase in the $\beta$ subunit at parturition. By using progesterone and onapristone, they were able to alter expression of the VDCC in a fashion consistent with a role of this ion channel in parturition. Thyroid hormone receptors are known to regulate gene expression. Thus, it is likely that the shift in potency to Ca$^{2+}$ antagonists observed in pregnant hypothyroid rat uterus was caused by expression of particular Ca$^{2+}$ channel subunit that has a reduced affinity to dihydropyridines. Logically, it is intriguing that candidates for these channel type could be either the...
subtypes of L-type $\text{Ca}^{2+}$ channel α1C [α1C,1.2] and α1D [α1C,1.3] or even the T-type $\text{Ca}^{2+}$ channel (α1C,3.2). These channel types exhibit IC$_{50}$ values for dihydropyridines of 0.1-1.4 nmol/l$^{53}$, about 0.2 μmol/l$^{54}$ and 3-5 μmol/l, respectively.

In conclusion, the present study on the rat uterus provides evidence for the first time that hypothyroidism reduces the spontaneous rhythmic contractions and the influx of Ca$^{2+}$ through voltage-dependent L-type Ca$^{2+}$ channels in patient with latent hyperthyroidism, and diltiazem. The decrease in L-type Ca$^{2+}$ channel blockers such as nifedipine and diltiazem. The decrease in L-type Ca$^{2+}$ channel function, however, should be confirmed directly by further studies using electrophysiological methods to measure directly ion currents and molecular analysis to identify the expression of α$\text{1}_{\text{C}}$ subunits that is being reduced in pregnant hypothyroid rat myometrium cells.

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