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Effects of Cyclobuxine D on Drug-Induced Contractions of the Isolated Rat Uterine Muscle and Potassium-Activated Calcium Channels in an Intestinal Smooth Muscle

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ABSTRACT

Cyclobuxine D, extracted from *Buxus microphylla* var. *koreana* Nakai, is a steroidal alkaloid. Many pharmacological effects of cyclobuxine D were examined in our Lab. Cyclobuxine D showed a significant bradycardic effect in the rat heart and an inhibitory action on acetylcholine and Ba^{++}-induced contraction of the longitudinal muscle isolated from the rabbit jejunum.

In this study, we investigated the effect of cyclobuxine D on the contractile response-elicited by acetylcholine, oxytocin and Ba^{++} in rat uterine. In order to analyse the inhibitory action of cyclobuxine D on the smooth muscle, we examined the inhibitory action of cyclobuxine D against the contractile response of the high potassium-depolarized rat ileum to calcium. Concentration-dependent decrease in the peak tension and duration of the acetylcholine, oxytocin and Ba^{++}-induced contraction in the isolated rat uterus was observed when cyclobuxine D was added to the organ bath.

The isolated longitudinal muscle from the rat ileum was immersed calcium-depleted potassium-depolarizing solution. Ten minutes after, 1.8 mM $CaCl_2$ was added to muscle bath and elicited a biphasic increase in muscle tension. Cyclobuxine D (6.2×10^{-5} M) produced an appreciable inhibition of both components of the mechanical response. In addition, 3.1×10^{-4} M cyclobuxine D, introduced at a point when the tonic response had reached its maximum level, caused the muscle to exhibit a rapid lose of tension. Based on these experimental results, we propose the possibility that the inhibitory action of cyclobuxine D on the acetylcholine, oxytocin and Ba^{++}-induced contraction in the isolated rat uterus may be due to blocking potassium-activated calcium channels, voltage-sensitive calcium channels.

Key Words: Cyclobuxine D, Oxytocin, Acetylcholine, Biphasic response, Potassium-activated calcium channel.

INTRODUCTION

Smooth muscle contraction is closely related to levels of intracellular free calcium. Intracellular free calcium levels are regulated by transport via calcium-channel (Bulbrign & Tomita, 1970; Burgen, 1970; Bolton, 1979; Kostyuk, 1980), calcium pump (Schatzmann, 1975), Na^+-Ca^{++}-exchange site (Blaustein, 1974; Grover *et al.*, 1983), uptake and release from sarcoplasmic reticulum, release from the binding site of cell membrane (Carafol & Crompton, 1978) and phosphatidylinositol turnover (Michell, 1975; Michell *et al.*, 1976). It has been shown that some drugs relaxed the smooth muscle due to a reduction of intracellular calcium concentration as a consequency of the inhibition of calcium influx through voltage or receptor-

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dependent calcium channels of the cell menbrane.

Lee *et al.*(1987) reported that cyclobuxine D, extracted from *Buxus microphylla* var *koreana* Nakai, inhibited the contractile responses-induced by acetylcholine and barium chloride in the isolated smooth muscle from the rabbit intestine. In this study, we investigated the effects of cyclobuxine D on the contractile responses-elicited by acetylcholine, oxytocin and barium. In order to analyse the inhibitory action of cyclobuxine D on the smooth muscle, we examined the inhibitory action of cyclobuxine D against the contractile responses of the high potassium-depolarized rat ileum to calcium.

MATERIALS AND METHODS

Uterine preparation

Female spraque-Dawley rats weighing 150-200 g were sacrificed by exsanguination and uterine horns with the ovarians were removed and dissected free from adjacent tissues. Strips of 2 cm in length were immersed in a 5 ml organ bath bubbled by air at 38±1℃ and containing a nutrient solution of the following composition (mM): NaCl, 154; KCl, 5.6; $CaCl_2$, 1.8; $NaHCO_3$, 2.4 and glucose 5.6, dissolved in distilled water. After 30 -60 minutes equilibration time, drug were injected into the bathing solution. The components that describe the contraction patterns were peak tension (P) and duration (D).

Ileum preparation

The longitudinal smooth muscle isolated from the rat ileum was used in this study. In each experiment, a segment of muscle approximately 3 cm long was suspended in a muscle bath that contained Tyrode's solution maintained 30±1℃. The Tyrode's solution had following composition (mM): NaCl, 137; KCl, 2.7; $CaCl_2$, 1.8; $MgCl_2$, 0.5; NaH_2Po_4, 0.3; $NaHCO_3$, 12 and glucose, 5.6, and bubbled with air. Other bathing solutions that were used in this investigation include: 1) a potassium depolarizing solution in which all the NaCl of the Tyrode's solution replaced by an equal molar concentration of KCl; 2) a calcium-depleted solution in which 1.8 mM $CaCl_2$ was not added to the Tyrode's solution; 3) a calcium-depleted potassium-depolarizing solution in which 1.8 mM $CaCl_2$ was not added to the potassium depolarizing solution.

Isometric contraction of the longitudinal muscle was measured by means of a Narco 7173 Myograph transducer and recorded on a Narco physiograph. Initial tension on the muscle was set at 0.5 g.

Drugs used were acetylcholine chloride (Sigma), oxytocin (Sigma), barium chloride (Santoku Chem. Co.) and cyclobuxine D (crystallized in our Lab.). All drugs were dissolved in the bathing solution. Drug concentrations described in this paper are expressed as final concentration in the organ bath.

The data obtained were expressed as mean± SEM. Student's t-test was used for statistical evaluation of the data.

RESULTS

A dose-dependent relaxation was observed when cyclobuxine D was added to the organ bath (Table1). Cyclobuxine D decreased significantly peak tension more than duration of the normal uterine contraction. Cyclobuxine D (6.2×10^{-4} M) decreased peak tension by 54 to 70% and duration by 12 to 44%, respectively.

The effect of cyclobuxine D on the peak tension (P) and duration (D) of the ACH-induced contraction

As the concentration of cyclobuxine D was increased from 6.2×10^{-6} to 6.2×10^{-4} M, the peak tension(P) and duration (D) of the ACH-induced contraction were significantly decreased (Fig. 1). The increase in P and D induced by ACH were completely inhibited in the presence of 6.2×10^{-4}

Table 1. Effects of cyclobuxine D on the longitudinal smooth muscle isolated from the rat uterine

Treatment	Conc. ($\times10^{-4}$ M)	Peak tension (cm)	Duration (cm)
Control	–	2.91 ± 0.32	0.68 ± 0.11
Cyclobuxine D	0.77	2.85 ± 0.25	0.69 ± 0.05
Cyclobuxine D	1.5	2.25 ± 0.19*	0.65 ± 0.08
Cyclobuxine D	3.1	1.61 ± 0.37**	0.56 ± 0.09
Cyclobuxine D	6.2	1.22 ± 0.34**	0.49 ± 0.11*

Each value indicates the mean ± SEM
Significant difference from control value (* $P < 0.05$, ** $P < 0.01$). In duration, 3 cm is equal to 1 min.

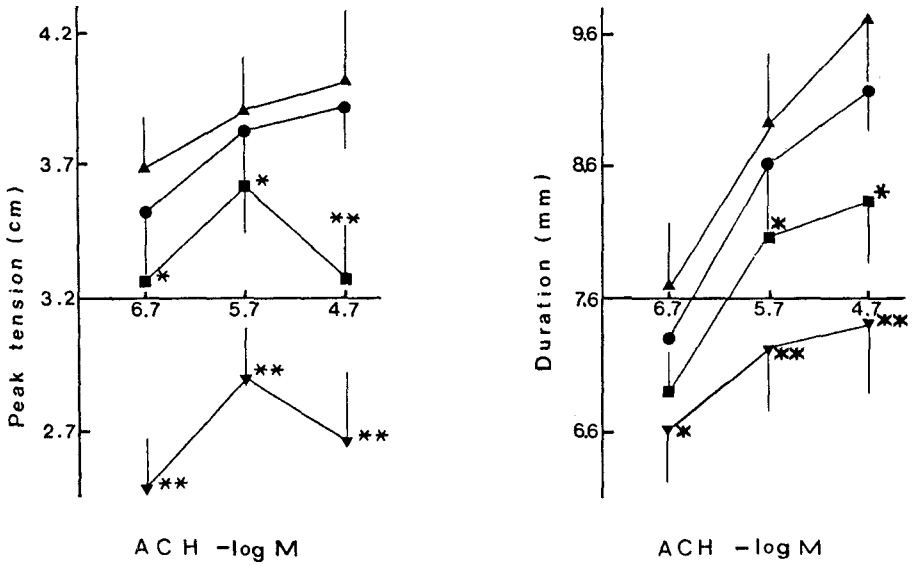

Fig. 1. The effect of cyclobuxine D on the peak tension (left panel) and duration (right panel) of the uterine contraction induced by acetylcholine. Each point is the mean ± SEM. The symbols correspond to the followings : ▲, control ; ●, 6.2×10^{-6} M ; ■, 6.2×10^{-5} M ; ▼, 6.2×10^{-4} M cyclobuxine D. Significant difference from control value (* $P < 0.05$, ** $P < 0.01$). In duration, 30 mm is equal to 1 min.

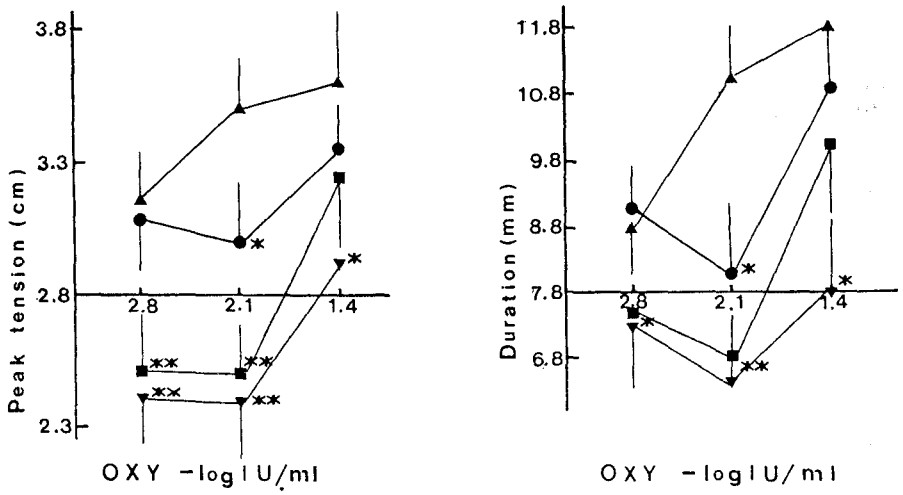

Fig. 2. The effect of cyclobuxine D on the peak tension (left panel) and duration (right panel) of the uterine contraction induced by oxytocin. Each point is the mean ± SEM. The symbols correspond to the followings : ▲, control ; ●, 1.5×10^{-4} M ; ■, 3.1×10^{-4} M ; ▼, 6.2×10^{-4} M cyclobuxine D. Significant difference from control value (* $P < 0.05$, ** $P < 0.01$). In duration, 30 mm is equal to 1 min.

M cyclobuxine D. Cyclobuxine D was more potent to inhibit the P than the D of the ACH-induced contraction (Fig. 1).

The effect of cyclobuxine D on the peak tension (P) and duration (D) of the OXT-induced contraction

As the concentration of cyclobuxine D was

Table 2. Inhbitory action of cyclobuxine D on the contractile response-induced by barium chloride in the longitudinal smooth muscle isolated from the rat uterine

Treatment	Conc ($\times 10^{-4}$ M)	Peak tension (cm)	Duration (cm)
Control	–	3.73 ± 0.35	0.85 ± 0.13
$BaCl_2$	0.41	4.28 ± 0.07*	1.37 ± 0.09**
Cyclobuxine D	1.5	4.09 ± 0.21	0.83 ± 0.09
Cyclobuxine D	6.2	3.62 ± 0.19**	0.67 ± 0.11**

Each value indicates the mean ± SEM
Significant difference from control value (* $P < 0.05$, ** $P < 0.01$). In duration, 3 cm is equal to 1 min.

increased 1.5×10^{-4} to 6.2×10^{-4} M, the P and D of the OXT-induced contraction were gradually decreased (Fig. 2). The P and D of the high OXT(0.2 IU/5 ml)-induced contraction was decreased by about 44% and 48% in the presense of cyclobuxine D ($3{,}2 \times 10^{-4}$ M), respectively. Cyclobuxine D was more potent to inhibit ACH than OXT-induced contractions.

The effect of cyclobuxine D on the peak tension (P) and duration (D) of the $BaCl_2$-induced contraction

Barium was more potent to increased the duration than peak tension of the rat uterine contraction (Table 2). Barium increased the peak tension and duration of the rat uterine contraction by about 15% and 61%, respectively. Cyclobuxine D (6.2×10^{-4} M) decreased significantly the increase in P and D of the barium-induced contraction.

The effect of cyclobuxine D on potassium-activated calcium channels in an isolated rat ileum

Longitudinal muscles of the rat-ileum were immersed in a calcium-depleted potassium-depolarizing solution for an additional 10 min preceding addition of 1.8 mM $CaCl_2$ to the muscle bath. The addition of the calcium immediately elicited the rapid, highly transient phasic portion of the tension response (Fig. 3, A). After some degree of relaxation occurred, the muscle underwent a second more gradual rise in tension, referred to as the tonic response (Fig. 3, B). The gradual decrease in magnitude of component B was followed

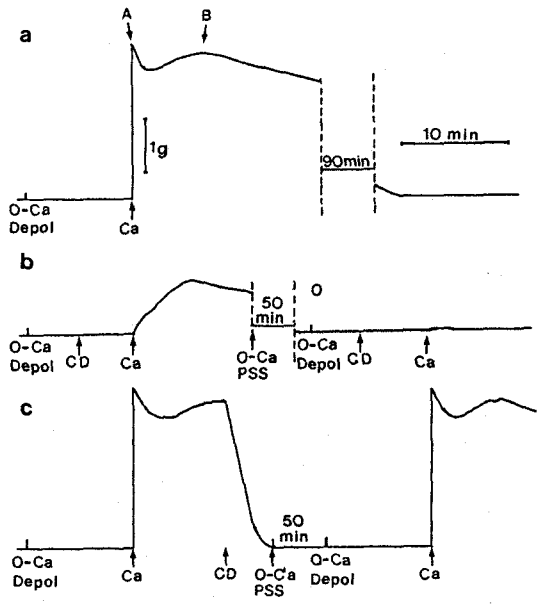

Fig. 3. Isometric contractions of the longitudinal muscle. At a, the recording represents a control contraction elicited by 1.8 mM $CaCl_2$ (Ca) after the muscle had been suspended in a calcium-depleted Tyrode's solution for 50 min and in a calcium-depleted potassium-depolarizing solution (O-Ca Depol) for an addition 10 min. At b, the recording shows the mechanical response developed by the muscle after it was subjected to the same treatment, but in addition 6.2×10^{-5} M and 3.1×10^{-4} M cyclobuxine D (CD) were added to the bathing medium 5 min before the 1.8 mM $CaCl_2$ was introduced. At c, 3.1×10^{-4} M cyclobuxine D (CD) was added to the muscle bath at the point at which the tonic response had reached its maximum tension. The term (O-Ca PSS) refers to the point at which the muscle was washed and reimmersed in a calcium-depleted Tyrode's solution for 50 min.

for 100–120 min. The development of these two types of tension changes in the presence of a high potassium ion concentration has been observed previously by numerous investigators (Imai and Takeda, 1967; Syson and Huddart, 1973; Triggle and Triggle, 1976; Gabella, 1978; Hurwitz *et al.*, 1980; Sally *et al*, 1985). Both components of contractile response were found to be sensitive to cyclobuxine D. In the presence of 6.2×10^{-5} M cyclobuxine D (Fig 3, b), phasic response (component A) was completely inhibited and tonic

response (component B) was inhibited over 70% (n=3). In the presence of 3.1×10^{-4} M cyclobuxine D (Fig 3, b), both components were completely inhibited. Furthermore, in another set of experiments, cyclobuxine D was added to the bathing medium shortly after component B reached its highest magnitude. Under these conditions, the muscle exhibited a rapid loss of tension (Fig. 3, c).

DISCUSSION

These present findings show that in longitudinal smooth muscle isolated from the rat uterine, cyclobuxine D inhibits dose-dependently the contractile response to acetylcholine, oxytocin and Ba^{++}. In ACH, OXT and Ba^{++}-induced contraction, cyclobuxine D decreased peak tension and duration dose-dependently. Cyclobuxine D was more potent to inhibit Ba^{++} and ACH than OXY-induced contraction.

There may be as many as four different sources of Ca^{++} for contraction: (a) calcium entering through the AP channel, (b) calcium entering through ROCs, (c) calcium bound to the receptors, (d) release of cacium from endoplasmic reticulum, inner surface of cell membrane, and possibly also mitochondria (Bolton, 1979). The muscarinic receptor activation increases the conductance of the cell membrane, which presumably indicates the opening of ROCs. In smooth muscle not generating AP, the opening of ROCs in the membrane may allow Ca^{++} to enter the cell, and the polarization that is produced probably contributes to the increase in PCa by opening more, potential-sensitive ion channels that admit Ca^{++} (Bolton, 1971; Bolton, 1972). Oxytocin produced contraction by inducing calcium influx through voltage-dependent channel (Barnes and Senior, 1985) and calcium release from cellular storage site (Batra, 1986). However, while action of acetylcholine depends mainly on the extracellular calcium or on a calcium pool loosely bound to the cellular membrane, the response to oxytocin seems to be dependent on the release of both tightly and loosely bound calcium (Calixto and Antonio, 1986). The shift to the right of the concentration-response curves to Ach and OXT in the presence of cyclobuxine D could indicate that in these conditions the compound is able to block the Ca^{++} channels couples to the respective receptors system for the agonist reducing the availability of Ca^{++} for contraction (Bolton, 1979; Cauvin *et al.*, 1983).

In another experiment, we investigated the inhibitory action of cyclobuxine D against the contractile responses of the high potassium-depolarized rat ileum to calcium. The isolated longitudinal muscle from the rat ileum was incubated a calcium-depleted solution for 50 min. It was then immersed in another calcium-depleted bathing medium in which all the sodium ions were replaced by potassium ions. Ten minutes after 1.8 mM $CaCl_2$ was added to the muscle bath and elicited a biphasic increase in muscle tension. Cyclobuxine D (6.2×10^{-5} M) produced an appreciable inhibition of both components of the mechanical response. In addition, 3.1×10^{-4} M cyclobuxine D, introduced at a point when the tonic response had reached its maximum level, caused the muscle to exhibit a rapid loss of tension.

In previous studies, many investigators showed that the isolated longitudinal muscle, when exposed to a high potassium bathing medium, will undergo a biphasic mechanical response (Harwitz *et al.*, 1980, 1982; Triggle and Triggle, 1976; Little *et al.*, 1985, Chang and Triggle, 1973). Little *et al.*, (1985) proposed that each of the two components of the mechanical response is dependent upon a different group of voltage-sensitive calcium channels in the longitudinal muscle.

The observations made in this study raise the possibility that the inhibitory action of cyclobuxine D on the acetylcholine, oxytocin and Ba^{++}-induced contraction in isolated longitudinal muscle may be due to blocking voltage-dependent calcium channels.

REFERENCES

Barnes NJG, Senior J: *The response of rat uterine tissue to oxytocin and prostaglandin $F_{2\alpha}$ in the presence of calcium channel antagonist. Br J Pharmacol 85 (supplement), p289, 1985*

Batra S: *Effect of oxytocin on calcium influx and efflux in the rat myometrium. Eur J Pharmacol 120:57-61, 1986*

Blaustein MP: *The interrelationship between sodium and calcium fluxes across cell membranes. Rev Physiol Pharmacol Biochem 70:33-82, 1974*

Bolton TB: *The effects of varying the concentrations of ions in the external solution of the oscillation of the membrane potential (slow wave) produced by carbachol in longitudinal ileal muscle.*

Pfluegers Arch. 335:85-96, 1972

Bolton TB: *On the nature of the oscillations of the membrane potential (slow wave) produced by acetylchoine or carbachol in intestinal smooth muscle. J Physiol 216:403-418, 1971*

Bolton TB: *Mechanisms of action of transmitters and other substances on smooth muscle. Physiol Rev 59:606-718, 1979*

Bulbring E, Tomita T: *Effects of Ca removal on the smooth muscle of the guinea-pig taenia coli. J Physiol London. 210:217-232, 1970*

Burgen ASV, Spero L: *Effects of calcium and magnesium on the response of intestinal smooth muscle to drugs. Br J Pharmacol 40:492-500, 1970*

Carafol E, Crompton M: *The regulation of intracellular calcium. Curr Top Membr Transp 10:151-216, 1978*

Cauvin C, Loutzenhiser R, Van Breemen C: *Mechanisms of calcium antagonist-induced vasodilation. Ann Rev Pharma Toxic 23:373-396, 1983*

Chang KJ, Triggle DJ: *Quantitative aspects of drug receptor interaction. I. Ca^{++} and cholinergic receptor activation in smooth muscle: A basic model for drug receptor interaction. J Theor Biol 40:125-154, 1973*

Gabella G: *Effect of potassium on the mechanical activity of taenia coli, uterus and portal vein of the guinea-pig. J Exp Physiol 63:125-146, 1978*

Grover AD, Kwan CY, Rangachari PK, Daniel EE: *Na-Ca exchange in a smooth muscle plasma membrane-enriched fraction. Am J Physiol 244: c158-c165, 1983*

Hurwitz L. Mcguffee LJ, Little SA and Blumberg H: *Evidence for two distinct types of potassium-activated calcium channels in an intestinal smooth muscle. J Pharm Exp Ther 214:574-580, 1980*

Hurwitz L, Mcguffee LJ, Smith PM, Little SA: *Specific inhibition of calcium channels by calcium ions in smooth muscle. J Pharmacol Exp Ther 220:382-388, 1982*

Imai S, Takeda K: *Actions of calcium and certain multivalent cations of potassium contracture of guinea-pig taenia coli. J Physiol 190:155-169, 1967*

Kostyuk PG: *Calcium ion channels in electrically excitable membranes. Neurosciences 5:945-959, 1980*

Lee JH, Park YH, Cho BH, Kim JB, Kim CM, Kim YJ, Kim CS, Cha YD, Kim YS: *Effects of cyclobuxine D on longitudinal smooth muscle isolated from the rabbit jejunum. New Med J 31: 49-53, 1988*

Little SA, Teaf E, Hurwitz L: *Cobalt-sensitive biphasic uptake of calcium ions in potassium-depolarized smooth muscle. J Pharmacol Exp Ther 232:746-753, 1985*

Michell RH: *Inositol phospholipids and cell surface receptor function. Biochem Biophys Acta 415:81-147, 1975*

Michell RH, Jafferji SS, Jones LM: *Receptor occupancy dose-response curve suggests that phosphatidylinositol break down may be intrinsic cholinergic receptor. FEBS Lett 69:1-5, 1976*

Schatzmann HJ: *Active calcium transport and Ca^{++}-activated ATPase in human red cells. Curr Top Membr Transp 6:126-168, 1975*

Syson AJ, Huddart H: *Contracture tension in rat vas deferens and ileal smooth muscle and its modification by external calcium and tonicity of the medium. Comp Biochem Physiol 45A:345-362, 1973*

Triggle CR, Triggle DJ: *An analysis of the action of cations of the lanthanide series on the mechanical responses of guinea-pig ileal longitudinal muscle. J Physiol 254:39-54, 1976*

=국문초록=

흰쥐 적출 자궁의 수축 작용과 흰쥐 장관에 있어 칼륨에 의해 활성화되는 칼슘 채널에 대한 Cyclobuxine D의 영향

순천향대학 의학부 약리학교실

권준택 · 이종화 · 박영현 · 조병헌 · 최규홍 · 김유재
김종배 · 김정목 . 김천숙 . 차영덕 . 김영석

Buxus microphylla var. *koreana* Nakai에 존재하는 steroidal alkaloid인 cyclobuxine D는 흰쥐에 있어 심박동수 감소 작용, 적출 개구리 심장에서 수축력 감소작용, 토끼 적출 장관에서 acetylcholine과 Ba^{++}에 유발되는 수축에 대한 억제작용 등을 나타낸다고 보고되었다.

본 연구에서는 흰쥐 적출 자궁에서 acetylcholine, oxytocin과 Ba^{++}에서 의해 나타나는 수축 작용에 대한 cyclobuxine D의 영향을 관찰하였으며, 또 흰쥐 적출장관에서 칼륨에 의해 활성화되는 칼슘채널에 대한 cyclobuxine D의 작용을 관찰하였다. Cyclobuxine D는 흰쥐 적출 자궁에서 acetylcholine, oxytocin과 Ba^{++}에 의해 증가되는 peak tension과 duration을 용량적으로 현저히 억제하였다. Cyclobuxine D는 oxytocin보다 acetylcholine에 의해 나타나는 수축작용에 대해 강하게 작용했다. 흰쥐 적출 장관(ileum)을 Ca을 고갈시킨 Tyrode's 용액에 40~50분 담그고 Na^+ 대신 K^+로 대체시킨 용액에 10분간 담근 후 1.8 mM $CaCl_2$를 가했을 때 이중적인 근육수축작용이 나타난다(Phasic and tonic increase in tension). Cyclobuxine D(6.2×10^{-5} M)은 이 두 components를 유의하게 억제하였으며 tonic component가 최대치에 도달했을 때 cyclobuxine D (3.1×10^{-4} M)을 가하면 근육은 긴장도를 빨리 상실했다. 이 결과는 적출 장관에 있어 칼륨에 의해 활성화되는 칼슘 채널이 cyclobuxine D에 의해 차단되고 있음을 나타낸다.

이상의 결과에서 cyclobuxine D의 흰쥐 적출 자궁에 대한 수축 억제 작용은 voltage-dependent calcium channel 차단에 밀접한 관련이 있는 것으로 사려된다.