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Effect of imidazole receptor active agents on porcine myometrial contractility

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돼지의 자궁근 수축성에 대한 몇가지 imidazole receptor active agents의 효과

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초 록 : 최근 동물의 진통 및 진정을 목적으로 널리 사용되고 있는 imidazole 유도체인 clonidine, medetomidine, etomidate 등의 약물과 xylazine의 효과를 발정정지기의 척출 돼지 자궁근에서 검토하였다. Clonidine(10^{-8}~10^{-6}M)이나 medetomidine(10^{-8}~10^{-6}M)은 xylazine과 비슷한 정도로 용량의존적인 자궁근의 수축을 일으켰다. Clonidine, medetomidine, xylazine 등의 EC_{50}는 각각 24.7nM, 19.9nM, 45.1nM이었다. 그러나 etomidate는 10^{-6}M 미만의 농도에서 반응이 거의 없었으며, 10^{-6}M 이상에서 수축반응을 일으켰다.

이들 agonists의 효과는 yohimbine(10^{-8}~10^{-6}M), idazoxan(10^{-7}~10^{-5}M), tolazoline(10^{-7}~10^{-5}M) 등의 a_2-adrenoceptor antagonists에 의해서 차단되었으나, a_1-adrenoceptor antagonist인 prazosin (10^{-6}M)에 의해서는 차단되지 않았다. 또한 Ca^{2+}-free medium이나 verapamil(10^{-5}M)의 전처치에 의해서 이들 agonist의 효과가 완전히 차단되었다.

결론적으로 발정정지기의 돼지 자궁근에서 clonidine, medetomidine, etomidate, xylazine 등은 a_2-adrenoceptors의 흥분을 통해 자궁근의 수축을 일으키며, 이 효과는 voltage-dependent Ca^{2+} channels을 통한 extracellular Ca^{2+} influx의 증가에 의한 것으로 추론하였다.

Key words : myometrium, clonidine, medetomidine, etomidate, xylazine.

Introduction

Clonidine, medetomidine, and etomidate are imidazole compounds and clonidine and medetomidine bind to a_2-adrenoceptors as well as non-adrenergic imidazole receptors [1-5]. These imidazole compounds can cause the effects that resemble those of xylazine, an a_2-adrenoceptor agonist. Xy-

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lazine, clonidine, medetomidine, and etomidate are frequently used as analgesics or sedatives in veterinary medicine[6~9]. These agents also cause various adverse effects such as bradycardia, heartblock, hypotension, ruminal atony, mydriasis, hyperglycemia, and abortion[10~13].

Agents which increase the myometrial contractility can be used to prevent the delayed parturition due to uterine inertia, especially in polytocous animals as sows, to reduce the postpartum bleeding, and to shorten the time of uterine involution. Both α_1- and α_2-adrenoceptors have been identified in the myometrial smooth muscles[14,15] and recent studies describe α_2-adrenoceptors as well as α_1-adrenoceptors may also mediate the increase in uterine contractility[16~18].

In the present study, we evaluated the effect of three imidazole compounds on myometrial contractility by comparing with taht of xylazine. We determined the difference of drug potency among agonists and also compared the potency of antagonists in blocking these effects of agonists. We also determined whether Ca^{3+}-free medium or verapamil, a voltage-dependent Ca^{2+} channel blocker, can abolish the effect of these agents.

Materials and Methods

Uterine strips : Uteri from sows were obtained from a local slaughter house. We used only uteri from the diestrous (days 12~15) period, which was determined through visual inspection of the ovary. They have the corpus luteum that were dark red to wine red at this stage[19]. Uteri were trimmed of connective tissue and endometrium and then stored in ice-cold Tyrode's solution that was aerated with 95% O_2 and 5% CO_2. The tissues were used for experiments within 10 hr of collection. Ages and breeds of the sows could not be determined.

Experimental condition : Longitudinal uterine strips (2cm × 0.2cm) were mounted in 10ml water-jacketed tissue baths. The tissue was maintained at 37℃ in the Tyrode's solution(137mM NaCl, 2mM KCl, 1mM $CaCl_2$, 0.4mM $MgCl_2$, 12mM $NaHCO_3$, 11mM dextrose, pH 7.4) aerated with a mixture of 95% O_2 and 5% CO_2. The Ca^{2+}-free Tyrode's solution was made by omitting 1mM $CaCl_2$ from the above solution. In the experiment using Ca^{2+}-free Tyrode's solution, we washed strips with a Ca^{2+}-free Tyrode's solution for three times and then bathed in this Ca $^{2+}$-free solution before the administration of the agonist. Agonist was given 10 min after the replacement with a Ca^{2+}-free Tyrode's solution.

The force developed by the strips were recorded through a Grass FT 03 force displacement transducer connected to a Grass Model 79 recorder. The contractile force was quantified by integrating the area under the tension versus time curve using a scanning program(SigmaScan, Jandel, Corte Madera, CA). All strips were initially exposed to hyperosmotic 140mM KCl for 2 min because this dose of KCl causes near maximal contractions. The contractile responses were expressed as the percentage of the response to hyperosmotic 140mM KCl. The experiments were performed under a resting tension of 2g and the strips were allowed to equilibrate for at least 30 min before experiments were begun and after 'washed out' during experiments. Agonist treatments were made at 10-min intervals in a cumulative dose schedule and only the 2-min maximal responses were compared. In all experiments, different uterine strips from the same uterus were randomly assigned to all treatment groups in one trial and one uterus was used for one trial only.

Drugs : The following drugs were purchased from Sigma Chemical Co. : xylazine HCl, clonidine HCl, prazosin HCl, yohimbine HCl, idazoxan HCl, tolazoline HCl, verapamil HCl and carbachol chloride. Medetomidine HCl and etomidate HCl were donated by Dr. W.H. Hsu. Iowa State University(Ames, IA).

Statistical analyses : Results were presented as mean ± SEM. Differences between means were determined by Student's *t*-test for grouped observations. The significance level was set at $p < 0.05$.

Results

Effects of agonists on myometrial contractility : Clonidine(10^{-8}~10^{-6}M) and medetomidine(10^{-8}~10^{-6}M) caused a dose-dependent increase in contractility of luteal phase myometrium and the contractile effect of these two im-

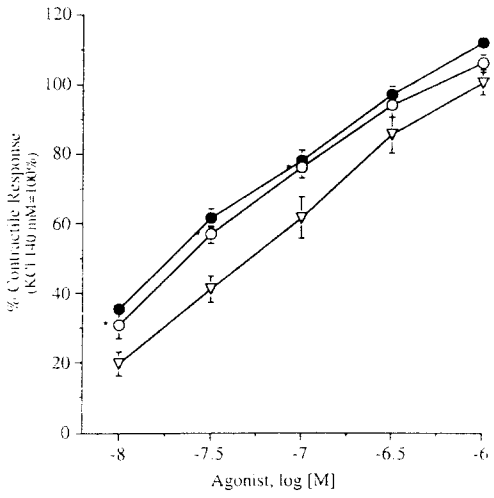

Fig 1. The contractile effects of clonidine(○), medetomidine (●), and xylazine(▽) in isolated porcine myometrial strips in the luteal phase of the estrous cycle(n=7.12), *p < 0.05), compared with the corresponding dose of xylazine.

idazole compounds were similar to that of xylazine(Fig 1). The EC_{50} of clonidine, medetomidine, and xylazine were 24. 7nM, 19.9nM, and 45.1nM, respectively.

Etomidate, interestingly, did not cause any increase in contractility in our present study at less than 10^{-6}M. However, a higher dose of etomidate(10^{-6}M) caused an increase in contractility(46.4±3.16, n=8).

Effects of antagonists on agonist-induced myometrial contractions : The effects of clonidine, medetomidine, and xylazine were inhibited by pretreatment with α_2-adrenoceptor antagonists such as yohimbine, idazoxan, and tolazoline. Yohimbine was most potent in antagonizing the effects of agonists in the present study(Figs 2~4). However, prazosin (10^{-6}M), an α_1-adrenoceptor antagonist, failed to block the effect of clonidine, medetomidine, and xylazine(Table 1). In addition, the effect of etomidate(43.7±6.1, n=3) was blocked by 10^{-7}M yohimbine(1.7±1.7, n=3), 10^{-6}M idazoxan(0, n =3), and 10^{-6}M tolazoline(0, n=3), but prazosin(10^{-6}M) did not block the effect of etomidate(44.6±4.8, n=3).

Effects of Ca^{2+}-free medium and verapamil on agonist-induced myometrial contractions : Ca^{2+}-free medium was used to determine if the effects of clonidine, medetomidine, etomidate, xylazine, and carbachol on myometrial contractions were mediated by extracellular Ca^{2+}, Ca^{2+}-free

Table 1. Effect of prazosin(10^{-6}M) on clonidine-, medetomidine-, etomidate-, and xylazine-induced increase in contractility in isolated porcine myometrial strips in the luteal phase of the estrous cycle(n=4)

Pretreatment	Agonist concentration, -log[M]				
	8	7.5	7	6.5	6
			clonidine		
None	30.8±3.6	56.8±2.6	76.1±3.7	94.3±3.7	106.2±2.4
prazosin	20.0±6.0	54.2±5.9	76.2±8.6	102.2±10.2	122.6±10.9
			medetomidine		
none	35.3±2.2	61.5±2.7	78.2±2.8	97.3±2.3	112.0±2.1
prazosin	37.3±4.6	59.4±4.4	74.3±4.2	89.1±2.8	103.9±2.7
			xylazine		
none	19.8±3.4	41.1±3.9	61.7±6.1	85.6±5.2	100.7±3.7
prazosin	31.0±7.6	53.6±7.0	76.2±4.8	95.4±12.1	106.5±7.9

Table 2. Effect of Ca^{2+}-free medium and verapamil(10^{-5}M) on clonidine(CLO)-, medetomidine(MED)-, etomidate(ETO)-, xylazine(XYL)-, and carbachol(CAR)- induced increase in contractility in isolated porcine myometrial strips in the luteal phase of the estrous cycle(n=4)

Pretreatment	Agonist(10^{-6}M)				
	CLO	MED	ETO	XYL	CAR
			Experiment A		
none	88.1±4.4	101.0±3.4	37.0±5.6	84.8±4.6	157.7±3.9
Ca^{2+}-free±0*	0*	0*	0*	0*	102.9±4.6*
			Experiment B		
none	106.8±8.4	102.4±3.4	59.9±3.7	89.4±5.3	147.2±8.7
verapamil	4.2±1.2*	1.0±1.0*	1.4±1.4*	2.6±1.6*	84.4±5.0*

*p < 0.05 : compared with the control(none) group.

medium completely prevented the contractile effects of clonidine, medetomidine, etomidate, and xylazine, but it significantly reduced the effect of carbachol(Table 2).

Verapamil(10^{-5}M) was used to determine whether this voltage-dependent Ca^{2+} channel blocker would abolish the effect of agonists. Verapamil completely prevented the contractile effects of clonidine, medetomidine, etomidate, and xylazine, but it significantly reduced the effect of carbachol (Table 2).

Discussion

Clonidine(10^{-8}~10^{-6}M) and medetomidine(10^{-8}~10^{-6}M) caused a dose-dependent increase in contractility of luteal phase myometrium. The contractile effects of these two imidazole compounds were similar to that of xylazine, even if there were little differences in the extent of the effects. The order of the potency was as follows : medetomidine 〉 clonidine 〉 xylazine. Although yohimbine, idazoxan and tolazoline are all selective α_2-adrenoceptor antagonists, yohimbine was most potent in antagonizing the effect of agonists in the present study. These are consistent with a previous report in which there was a differential ability of these antagonists in blocking the myometrial contractility of xylazine in sows. Yohimbine was approximateldy 10 times more potent than idazoxan in antagonizing the effect of xylazine in porcine uterine strips[20]. There, however, was an opposite report, in bovine uterine strips, in which yohimbine was approximately 50 times less potent than idazoxan in an-

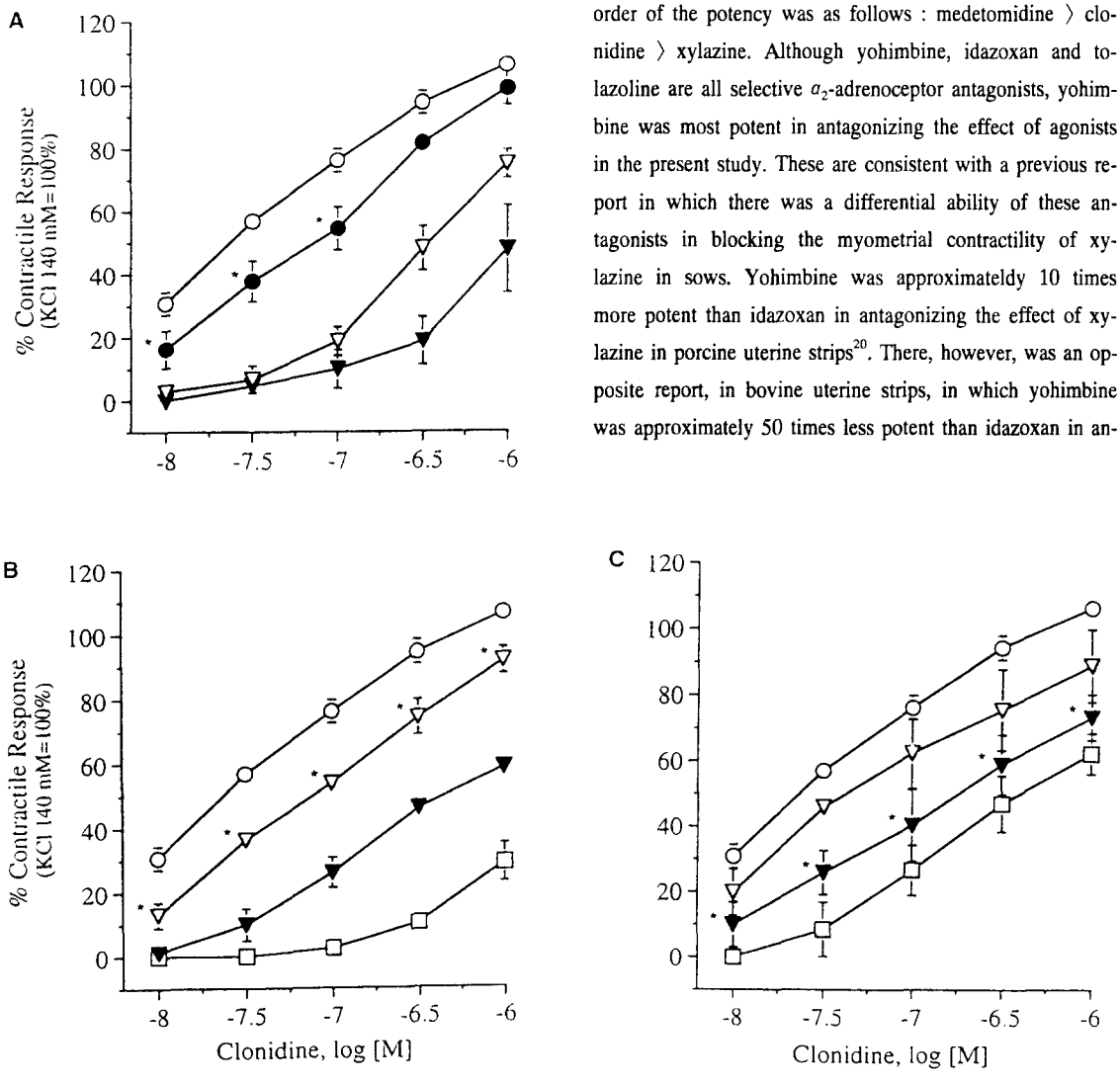

Fig 2. Effect of yohimbine(A), idazoxan(B), and tolazoline(C) on clonidine-induced increase in contractility of isolated porcine myometrial strips in luteal phase of the estrous cycle(n=4-13). Effects are shown in the absence(○) and in the presence of antagonists(10^{-8}M, ●; 10^{-7}M, ▽; 10^{-6}M, ▼; 10^{-5}M, □). *p 〈 0.05, compared with the control group at the corresponding dose of clonidine.

tagonizing the efect of xylazine[21]. It would be interesting to study why there are such differences among species.

On the other hand, prazosin(10^{-6}M), an α_1-adrenoceptor antagonist, failed to block the contractile effects of these agonists. Our results are consistent with those of a previous report indicating that α_2-adrenoceptors during the luteal phase of the estrous cycle account for more than 99% of α-adrenoceptors in porcine myometrium[22].

Etomidate, at less than 10^{-6}M, did not cause any increase in contractility in our present study. A higher dose of etomidate(10^{-6}M), however, caused an increase in contractility. In addition, the effect of etomidate was blocked by all α_2-adrenoceptor antagonists we used, but prazosin(10^{-6}M), the α_1-antagonist, did not block the effect of etomidate. Etomidate activates GABA-gated Cl-channels to induce its CNS effects[23]. However, there is no report regarding activation of α_2-adrenoceptors by etomidate. It would be interesting to study whether high dose of etomidate can also bind to α_2-adrenoceptors.

Although clonidine and related imidazole compounds can also bind to imidazole receptors in addition to α_2-adrenergic binding[4,24,25], in our present study, clonidine, medetomidine and etomidate caused an increase in myometrial contractility through the activation of α_2-adrenoceptors. Intracellular free Ca^{2+} concentration could be involved in the mediation of myometrial contractility[26,27]. Thus, to evaluate the role of extracellular and intracellular Ca^{2+}, we determined whether Ca $^{2+}$-free medium or verapamil, a voltage-dependent Ca^{2+} channel blocker[28], can abolish the effect of agonists. The Ca^{2+}-

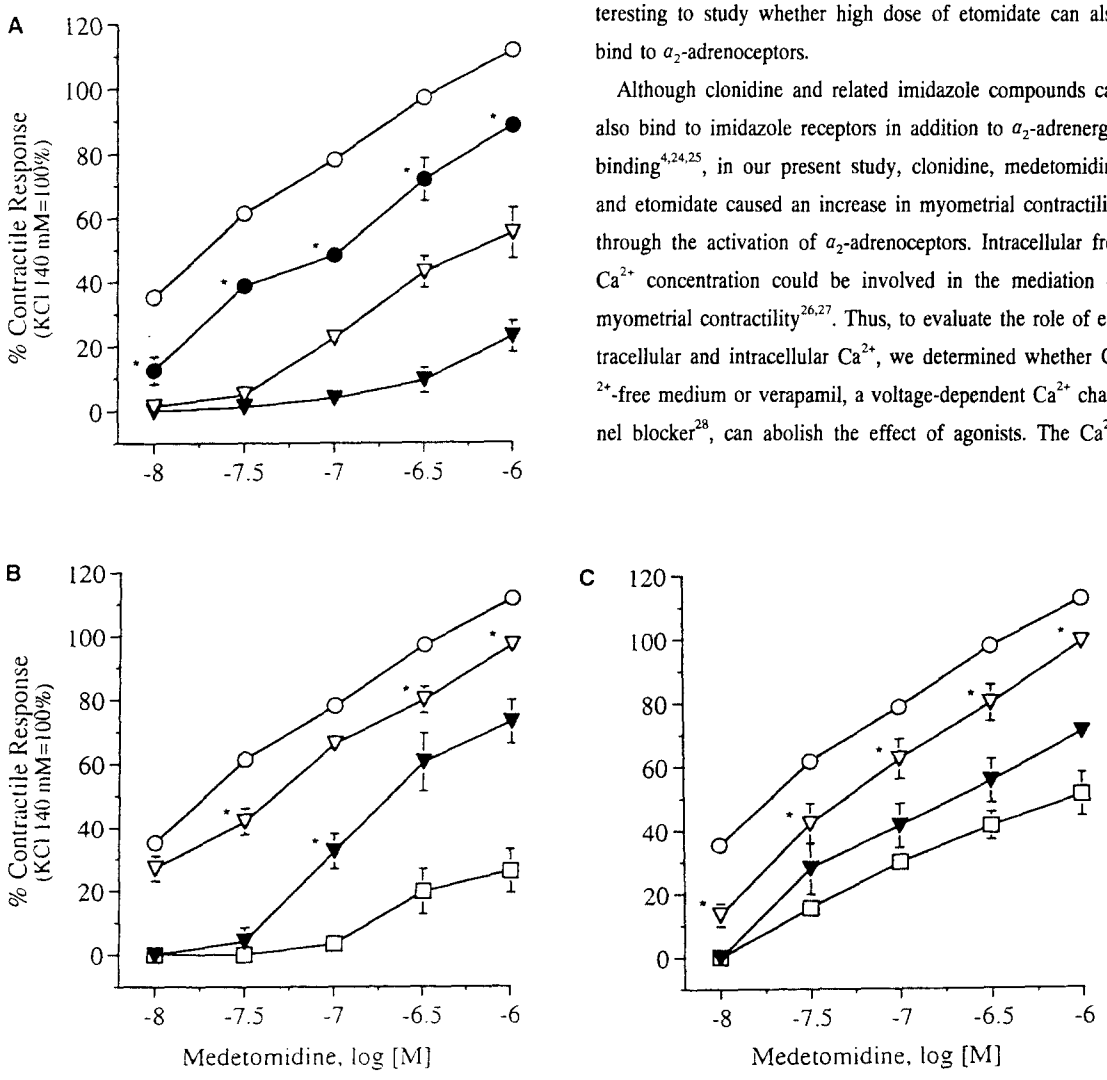

Fig 3. Effect of yohimbine(A), idazoxan(B), and tolazoline(C) on medetomidine-induced increase in contractility of isolated porcine myometrial strips in luteal phase of the estrous cycle(n=4-10). Effects are shown in the absence(○) and in the presence of antagonists(10^{-8}M, ●; 10^{-7}M, ▽; 10^{-6}M, ▼; 10^{-5}M, □). *$p < 0.05$, compared with the control group at the corresponding dose of clonidine.

free medium and verapamil, respectively, abolished the effect of clonidine, medetomidine, etomidate, and xylazine on myometrial contractility but only reduced that of carbachol. Our present results suggest that clonidine, medetomidine, etomidate, and xylazine induced the myometrial contractions through exclusively the extracellular Ca^{2+} influx by opening voltage-dependent Ca^{2+} channels.

Xylazine and imidazole compounds are frequently used as analgesics and sedatives in veterinary medicine. These agents can also cause various adverse effects and one of these adverse effects may be abortion, because these agents can increase the myometrial contractility. The present results, therefore, may be of clinical significance because these imidazole compounds and xylazine can modulate the myometrial contractility. Furthermore, the selective antagonists to block the effect of these agents in the uterus can be used to control the untoward reactions such as abortion by use of these agonists.

Conclusion

In the present study, we evaluated the effect of imidazle compounds on myometrial contractility, in luteal phase, by comparing with that of xylazine. The results of the present study suggest that clonidine, medetomidine, etomidate, and xylazine induced the porcine myometrial contractions through the activation of α_2-adrenoceptors, and through exclusively the extracellular Ca^{2+} influx by opening voltage-dependent Ca^{2+} channels.

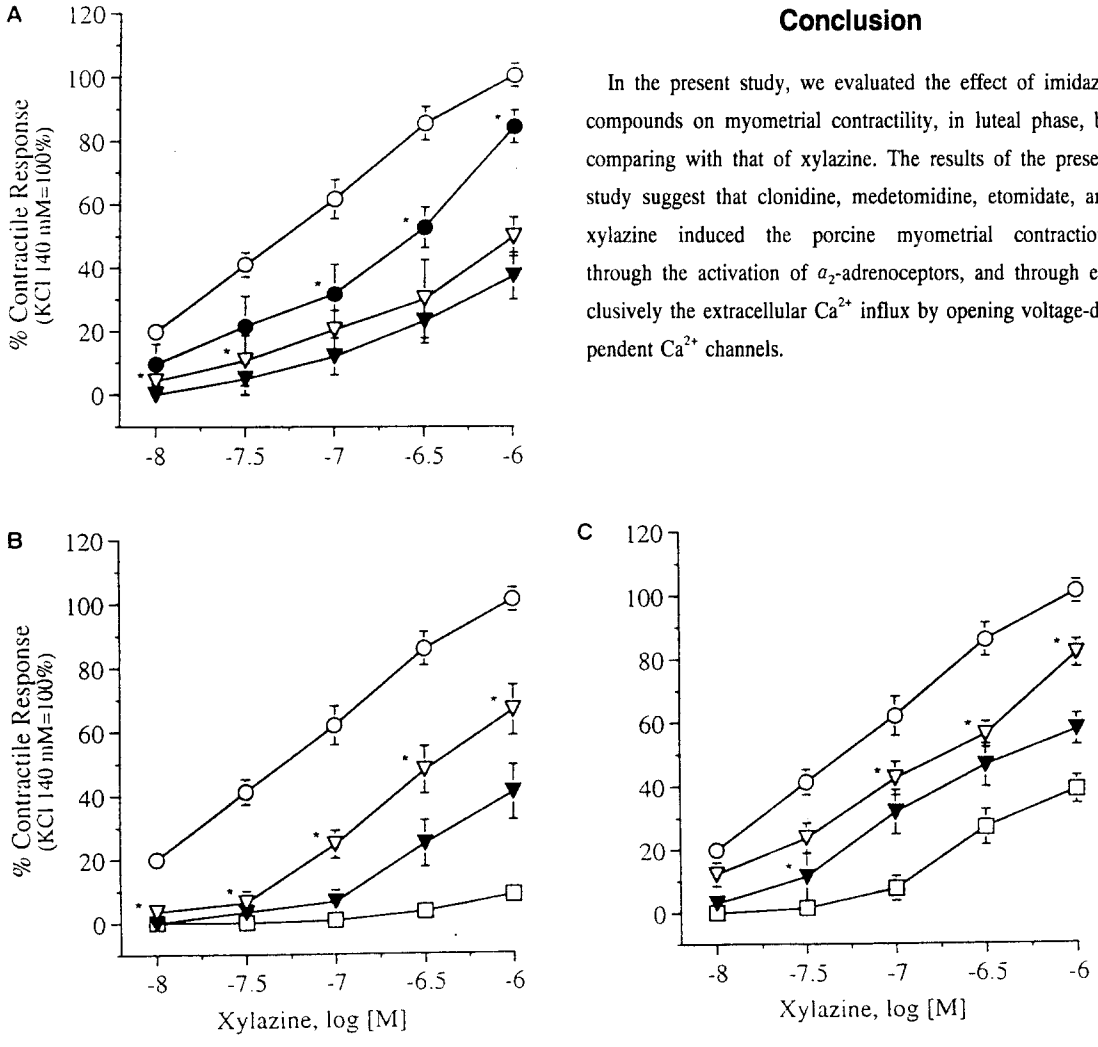

Fig 4. Effect of yohimbine(A), idazoxan(B), and tolazoline(C) on xylazine-induced increase in contractility of isolated porcine myometrial strips in luteal phase of the estrous cycle(n=4-12). Effects are shown in the absence(○) and in the presence of antagonists(10^{-8}M, ●; 10^{-7}M, ▽; 10^{-6}M, ▼; 10^{-5}M, □). *$p < 0.05$, compared with the control group at the corresponding dose of clonidine.

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