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The responsiveness to amitraz in isolated porcine myometrial strips

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척출 돼지 자궁근의 amitraz에 대한 반응성

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초록 : 수의학 및 농업분야에서 널리 사용되고 있는 살충제인 amitraz의 효과를 척출 돼지 자궁근에서 검토하였다. Amitraz(10^{-8}~10^{-6} M)는 발정정지기의 자궁근 수축성을 용량의존적으로 증가시켰다. Amitroaz의 이 효과는 yohimbine(10^{-8}~10^{-7} M)에 의해 용량의존적으로 차단되었으나, prazosin(10^{-6}M)에 의해서는 억제되지 않았다. 또한 Ca^{2+}-free Tyrode's solution에서나 verapamil(3×10^{-5} M)의 전처치에 의해서 amitraz의 수축효과는 완전히 억제되었다. 따라서 amitraz에 의한 발정정지기 돼지 자궁근의 수축은 α_2-adrenoceptor가 중개하며 이 효과는 주로 Ca^{2+}entry blocker에 감수성이 있는 Ca^{2+} channel을 통한 extracellular Ca^{2+} influx의 증가에 의한 것으로 여겨진다.

Key words : amitraz, myometrium, α_2-adrenoceptor, Ca^{2+} channel.

Introduction

Both α_1- and α_2-adrenoceptors have been identified in the myometrium using radioligand binding techniques.[1~4] It is generally believed that α_1- but not α_2-adrenoceptors mediate myometrial contractions. However recent studies suggest that α_2-adrenoceptors may also mediate the increase of uterine contractility.[5~7]

Amitraz(N'-(2,4-dimethylphenyl)-N- [((2,4-dimethylphenyl)imino)methyl] -N-methylmethanimidamide)is a formamidine insecticide/acaricide widely used in agriculture and veterinary medicine.[8~10] In veterinary medicine it has been used to control cattle ticks and to treat demodectic mange in dogs[11] and in agriculture as an orchard spray. Amitraz poisonings in humans as well as animals have been reported. It can produce side effects that resemble those of the α_2-adrenoceptor agonists xylazine and clonidine.[12,13] The most prominent toxic effects of amitraz in mammals are central nervous system depression, bradycardia, hyperglycemia, mydriasis, vomiting, and bloat. Moreover recent evidence suggests that α_2-adrenoceptors may mediate the pharmacological actions of amitraz.[11~16]

Therefore, the present study was designed to examine the responsiveness to amitraz in isolated porcine uterine strips during the diestrous stage.

Cytosolic-free Ca^{2+} concentration may be involved in the mediation of myometrial contractions.[17,18] Thus we also determined whether Ca^{2+}-free medium or a voltage-dependent Ca^{2+} channel blocker verapamil can abolish the effect of amitraz.

Materials and Methods

Uterine preparation : Uteri from sows were obtained

at a local slaughter house. Uteri were determined to be diestrous(days 12～15) through visual inspection of the ovary containing the corpus luteum, which at this stage were dark red to wine red.[19] Ages and breeds of the sows could not be determined. Uteri were trimmed of connective tissues and endometrium and then stored in Tyrode's solution(4℃ and aerated with 95% O_2, 5% CO_2) and used for experiments within 24 h.

Experimental condition : Approximately 2cm × 0.2cm longitudinal uterine strips were mounted in a 10mℓ water-jacketed tissue bath. The tissue was maintained at 37℃ in Tyrode's solution(137mM NaCl, 2 mM KCl, 1 mM $CaCl_2$, 0.4 mM $MgCl_2$, 11 mM dextrose, 12 mM $NaHCO_3$, pH 7.4) and the solution was aerated with a mixture of 95% O_2 and 5% CO_2. The experiments were performed under a resting tension of 2g and the tissues were allowed to equilibrate for at least 30 min before experiments were begun. The force developed by the tissue strips were recorded through a Grass FT 03 force displacement transducer connected to a Beckman model R 411 recorder and then contractile force was quantified by integrating the area under the tension vs. time curve. All strips were initially exposed to 10^{-6}M xylazine for 2min, because this dose of xylazine causes near maximal contractions. The maximal contraction was reached within 10 min after agonist administration. Agonist treatments were made at 10min intervals in a cumulative dose schedule and only the 2min maximal responses were compared. Results were expressed as the percentage of response to 10^{-6} M xylazine.

Drugs : The following drugs were used in the present study : amitraz(Upjohn), xylazine HCl(Mobay), verapamil HCl, carbachol chloride and yohimbine HCl(Sigma), and prazosin HCl(Pfizer). All drugs were dissolved in physiological saline except that xylazine, prazosin and amitraz were dissolved in 8.5% lactic acid to make 10^{-3} M solutions and pH was adjusted to pH 7.3 with NaOH. These solutions were further diluted with physiological saline to make appropriate doses.

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

Results

Effect of amitraz on myometrial contractility : Amitraz(10^{-8}～10^{-5} M) caused a dose-dependent increase of myometrial contractility in the diestrous stage(Table 1).

The effect of amitraz was significantly reduced in a dose-dependent manner by the pretreatment with yohimbine (10^{-8}～10^{-7}M), α_2-adrenoceptor antagonist(Table 2), but pretreatment with prazosin(10^{-6} M), α_1-adrenoceptor antagonist, did not affect the effect of amitraz(Table 3).

Table 1. Effect of amitraz on the contractility of isolated uterine strips in diestrous stage

n	Concentration of amitraz(M)			
	10^{-8}	10^{-7}	10^{-6}	10^{-5}
7	29.6±6.3	75.1±9.5	115.4±14.0	76.7±13.6

* Values are expressed as the percentage of response to 10^{-6} M xylazine.

Table 2. Effect of yohimbine (YOH) on amitraz-induced contractility in isolated diestrous stage uterine strips

Pretreatment	n	Concentration of amitraz(M)		
		10^{-8}	10^{-7}	10^{-6}
None	5	53.1±6.3	107.8±5.3	137.4±11.7
YOH(10^{-8} M)	5	13.5±8.3*	62.4±11.5*	104.9±14.8
YOH(3×10^{-8} M)	5	0*	7.8±6.0*	57.0±11.6*
YOH(10^{-7} M)	5	0*	0*	11.9±9.2*

* $p<0.05$ compared with non-pretreatment amitraz response.

Table 3. Effect of prazosin(PRA) on amitraz-induced contractility in isolated diestrous stage uterine strips

Pretreatment	n	Concentration of amitraz(M)		
		10^{-8}	10^{-7}	10^{-6}
None	4	12.8±7.7	64.1±6.2	102.4±9.8
PRA(10^{-6} M)	4	4.7±4.7	64.0±5.8	109.2±9.3

Table 4. Effect of Ca^{2+}-free medium on amitraz(AMI) and carbachol(CAR)-induced contractility in isolated diestrous stage uterine strips

Pretreatment	n	Agonists(10^{-6} M)	
		AMI	CAR
Control	4	108.5±16.5	186.2±19.3
Ca^{2+}-free	4	0*	77.8±9.4*

*$p<0.05$ compared with control.

Table 5. Effect of verapamil (VER) on amitraz(AMI) and carbachol(CAR)-induced contractility in isolated diestrous stage uterine strips

Pretreatment	n	Agonists(10^{-6} M)	
		AMI	CAR
Control	4	100.3±10.3	148.0±14.2
VER(3×10^{-5} M)	4	0*	30.9±5.2*

*$p<0.05$ compared with control.

Effects of Ca^{2+}-free medium or verapamil on amitraz- and carbachol-induced myometrial contractions : Ca^{2+}-free medium completely prevented the amitraz(10^{-6} M)-induced myometrial contractions but it only reduced the effect of carbachol(10^{-6} M), significantly(Table 4).

Verapamil(3×10^{-5} M), a voltage-dependent Ca^{2+} channel blocker, also completely blocked the effect of amitraz(10^{-6} M) but the effect of carbachol(10^{-5} M) was only decreased significantly(Table 5).

Discussion

Amitaz($10^{-8}\sim10^{-6}$ M) caused a dose-dependent increase of myometrial contractility. The α_2-adrenoceptor antagonist yohimbine($10^{-8}\sim10^{-7}$ M), but not an α_1-adrenoceptor antagonist prazosin(10^{-6} M), blocked the effect of amitraz in a dose-dependent manner. These are the evidence that amitraz caused a myometrial contractions mediated by α_2- but not α_1-adrenoceptors in porcine uterine strips. However, when the dose of amitraz were increased to 10^{-5} M the responses were decreased than that of 10^{-6}M. This is an very interesting find. Further work is needed to determine this.

Both α_1- and α_2-adrenoceptors have been identified in the myometrium using radioligand binding techniques in rats,[4] rabbits,[20] sheep,[21] pigs[2], and humans.[1] It is generally believed that α_1- but not α_2-adrenoceptors mediate myometrial contractions.[20] However recent evidence suggests that α_2-adrenoceptors may also mediate the increase of uterine contractility. Ko et al.[7] have found that xylazine caused a dose-dependent increase of uterine contractility in bovine and porcine uterine strips in which this effect were blocked by the α_2-adrenoceptor antagonists yohimbine and idazoxan, but not by the α_1-adrenoceptor antagonist prazosin. Moreover there is little information concerning the responsiveness to α_2-adrenoceptors in myometrium during the different stages of the estrous cycle. Therefore we also need to compare the effects of amitraz on porcine myometrial contractility during both the diestrous and estrous stage if further work could be planned.

Although there is little evidence concerning the effect of myometrial contractions on cytosolic-free Ca^{2+} regulation. cytosolic-free Ca^{2+} may has a major role in the mediation of myometrial contractions. When the uterine strips were pretreated with Ca^{2+}-free medium the effect of amitraz on myometrial contractility was completely inhibited but that of carbachol was only partly reduced. Moreover verapamil, a voltage-dependent Ca^{2+} channel blocker, completely blocked the effect of amitraz but only moderately reduced that of carbachol. These results suggest that amitraz induces the myometrial contractions by opening voltage-dependent Ca^{2+} channels to increase Ca^{2+} entry, whereas carbachol induces its effect by increasing the release of Ca^{2+} from intracellular pools as well as by opening Ca^{2+} channels.

The results of present study showed that amitraz-induced myometrial contractions during the diestrous stage is mediated by α_2-adrenoceptors and this effect is mainly mediated by extracellular Ca^{2+} influx through Ca^{2+} channels sensitive to Ca^{2+} entry blockers.

Conclusion

The effects of amitraz on porcine myometrial contractility were studied using isolated uterine strips during the diestrous stagte. Amitraz(10^{-8}~10^{-6} M) caused a dose-dependent increase of myometrial contractility in the diestrous stage. The α_2-adrenoceptor antagonist yohimbine(10^{-8}~10^{-7} M), but not an α_1-adrenoceptor antagonist prazosin(10^{-6} M), blocked the effect of amitraz in a dose-dependent manner. When the uterine strips were pretreated with Ca^{2+}-free Tyrode's solution of verapamil(3×10^{-5} M) the effects of amitraz were completely abolished, whereas those of carbachol were only moderately reduced.

The results suggest that the amitraz-induced myometrial contractions during the diestrous stage is mediated by α_2-adrenoceptors and this effect is mainly mediated by extracellular Ca^{2+} influx through Ca^{2+} channels sensitive to Ca^{2+} entry blockers.

References

1. Bottari SP, Vokaer A, Kaivez E, et al. Identification and characterization of α_2-adrenergic receptors in human myometrium by [^{3}H] rauwolscine binding. *Am J Obstet Gynecol* 1983 ; 146 : 639~643.
2. Rexroad CE, Guthrie HD. Alpha adrenergic receptors in myometrium of pregnant and nonpregnant pigs until Day 19 postestrus. *Biol Reprod* 1983 ; 29 : 615~619.
3. Maltier JP, Legrand C. Characterization of α-adrenoceptors in myometrium of preparturient rats. *Europ J Pharmacol* 1985 ; 117 : 1~13.
4. Kyozuka M, Crankshaw DJ, Crankshaw J, et al. Alpha-2 adrenoceptors on nerves and muscles of rat uterus. *J Pharmacol Exp Ther* 1988 ; 244 : 1128~1138.
5. Mamet PG, Garcia-Villar R, Laurentie MP, et al. *In vivo* pharmacological characterization of alpha adrenergic receptors in sheep myometrium and their physiological meaning. *Biol Reprod* 1987 ; 37 : 241~248.
6. Rodriguez-Martinez H, Mckenna D, Weston PG, et al. Uterine motility in the cow during the estrous cycle. III. Effects of oxytocin, xylazine, and adrenoceptor blockers. *Theriogenology* 1987 ; 27 : 359~368.
7. Ko JCH, Hsu WH, Evans LE. The effects of xylazine and alpha-2 adrenoreceptor antagonists on bovine uterine contractility *in vitro*. *Theriogenology* 1990 ; 33 : 601~611.
8. Cannon W. Amitraz in treatment of canine demodicosis. *Mod Vet Pract* 1983 ; 64 : 899~900.
9. Folz SD. Demodicosis(Demodex canis). *Comp Cont Ed Pract Vet* 1983 ; 5 : 116~121.
10. Muller GH. Amitraz treatment of demodicosis *J Amer Anim Hosp Assn* 1983 ; 19 : 435~441.
11. Farmer H, Seawright AA. The use of amitraz(N-2,4(dimethylphenyl)-N- [((2,4-dimethylphenyl)imino)-N-methylmethanidamide]) in demodicosis in dogs. *Austr Vet J* 1980 ; 57 : 537~541.
12. Dobozy VS. Mitaban safety. *DVM* 1982 ; 13(No. 11~12) : 54~55.
13. Hsu WH, Kakuk TJ. Effects of amitraz and chlordimeform on heart rate and pupil diameter in rats : Mediated by α_2-adrenoceptors. *Toxicol Appl Pharmacol* 1984 ; 73 : 411~415.
14. Schaffer DD, Hsu WH, Hopper DL. The effects of yohimbine and four other antagonists on amitraz-induced depression of shuttle avoidance responses in dogs. *Toxicol Appl Pharmacol* 1990 ; 104 : 543~547.
15. Koeiman NRL, Hsu WH. Interaction between amitraz and α_2-adrenoceptors inhibits epinephrine-induced canine platelet aggregation. *Arch Int Pharmacodyn* 1991 ; 310 : 56~65.
16. Pass MA, Mogg TD. Effect of amitraz and its metabolites on intestinal motility. *Comp Biochem Physiol* 1991 ; 99C : 169~172.
17. Anwer K, Sanborn BM. Changes in intracellular free calcium in isolated myometrial cells : role of extracellular and intracellular calcium and possible involvement of guanine nucleotide-sensitive proteins. *Endocrinology* 1989 ; 124 : 17~23.
18. Molnar M, Hertelendy F. Regulation of intracellular free calcium in human myometrial cells by prostaglandin $F_2\alpha$: Comparison with oxytocin. *J Clin Endocrinol Metab* 1990 ; 71 : 1243~1250.
19. Arthur GH, Noakes DE, Pearson H. *The estrous cycle and its control. In : Veterinary Reproduction and Obstetrics(Theriogenology).* 6th ed. London : Bailliere Tindall, 1989 ; 25~26.
20. Hoffman BB, Lavin TN, Lefkowitz RJ, et al. Alpha adrenergic receptor subtypes in rabbit uterus : medi-

ation of myometrial contraction and regulation by estrogens. *J Pharmacol Exp Ther* 1981 ; 219 : 290~295.
21. Rexroad CE. Binding of dihydroalprenolol and dihydroergocryptine to sheep myometrium. *Biol Reprod* 1981 ; 24 : 831~838.