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Studies on the pesticide amitraz-induced bradycardia

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Abstract : In veterinary medicine amitraz has been used as an insecticide to eliminate mites, lice, and ticks in dogs, cats, goats, swine and cattle.

We performed the experiment on bradycardiac effect of amitraz in rabbits. Under anesthetized with urethane (1 g/kg, 25% w/v, SC), the femoral artery was cannulated by a polyvinyl tube which was connected with pressure transducer for continuous measurement of heart rate. Amitraz (0.5-2.5mg/kg, IV) reduced the heart rate in a dose-dependent manner. Pretreatment of yohimbine (2.0 mg/kg) or atropine (2.0mg/kg and 4.0mg/kg) affected the response of amitraz but pretreatment of prazosin (0.5mg/kg) or propranolol (1.0mg/kg) did not. Moreover, the effect of amitraz was blocked by vagotomy.

The result of our experiment suggest that amitraz may reduce the heart rate by increasing acetylcholine through activating vagal nerves and α_2-adrenoceptors could be involved in activating vagal nerves.

Key words : amitraz, heart rate, bradycardiac effect.

Introduction

Amitraz (N'-2,4-(dimethylphenyl)-N-[{(2,4-dimethylphenyl) imino} methyl]-N-methylmethanimidamide) is an acaricide classified as formamidines and is widely used in agriculture and veterinary medicine[1,2].

In veterinary medicine it has been used to eliminate mites, lice, and ticks in dogs[3,4], cats[5], goats[6], swine, and cattle[7].

Amitraz poisonings in humans as well as animals have been reported. The most prominent toxic effects of amitraz in mammals are central nervous system depression, bradycardia, hyperglycemia, mydriasis, vomiting, and bloat. Several adverse effects of amitraz resemble those of the α_2-adrenoceptor agonists[8,9]. Moreover recent evidence suggests that α_2-adrenoceptors may be mediated in the action of ami-

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traz[10].

The purpose of present study was to investigate the possible mechanisms of receptors involved in the bradycardiac effect of amitraz in rabbits.

Materials and Methods

Animals : Albino rabbits of either sex with body weight ranging from 1.5 to 2.0kg were used. These animals were kept under the conditions of constant temperature maintained at 22~25℃, and illumination (12hr light-dark cycle). These animals have free access to food and water all the time.

Chemicals : Atropine, yohimbine, prazosin, and propranolol were dissolved in saline (All chemicals were purchased from Sigma Co.). Amitraz were dissolved in DMSO (10mg of amitraz/ml of DMSO). These solutions were further diluted with physiological saline to make appropriate doses. All antagonists were dissolved in saline immediately before use and injected at 10~15 minutes before amitraz administration.

Experimental procedures : Rabbits were anesthetized with urethane (1 g/kg, 25% w/v, SC). Following cannulation of the trachea, the femoral artery was cannulated by a polyvinyl tube. The cannular was connected to Statham P 23XL pressure transducer (Ohmeda Inc.) for continuous measurement of heart rate (HR). All responses were recorded on a Grass model 79 recorder. Control experiments were performed to examine the effects of DMSO (0.2ml/kg, IV), as a vehicle, but DMSO did not produce any effect in HR in rabbits. Then after an initial 30-min resting period, continuous doses of amitraz were administrated every 40 min intervals into ear vein. The doses of amitraz were 0.5, 1.0, 1.5, 2.0, and 2.5mg/kg. Heart rate was measured at 0.5, 1, 3, 5, 10, 20, and 40 mins after each injection.

Then we investigated time-course effects of the changes of amitraz in HR after pretreatment of each antagonist; prazosin as an α_1-adrenergic antagonist, yohimbine as an α_2-adrenergic antagonist, propranolol as a β-adrenergic antagonist, and atropine as a cholinergic antagonist and compared with the effects of amitraz (2.0mg/kg, IV) alone. For more information about the cholinergic nerve involvement in the bradycardiac effect of amitraz, we performed vagotomy over the neck. Results were showed by % inhibition to the heart rate before agonist administration.

All values of heart rate were expressed as mean±SEM and statistical significance among groups was analyzed by Student's *t*-test. The significance level was set at $p < 0.05$.

Results

Effect of amitraz on heart rate : Amitraz reduced heart rate in the dose range of 0.5~2.5mg/kg. The reduction began at 0.5mg/kg and showed the greatest bradycardiac effect at 2.5mg/kg. However, even at the highest dose these reductions did not exceed 50% of inhibition. The inhibitory effects of amitraz were increased dose-dependently(Fig 1).

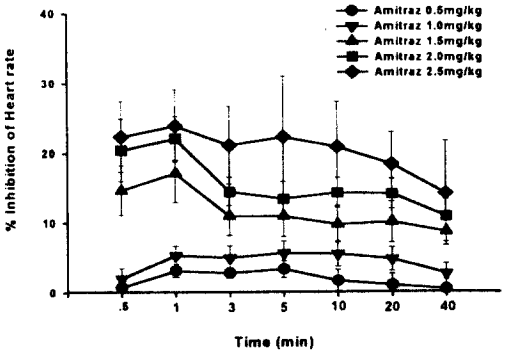

Fig 1. Dose-response relationship of amitraz (IV). Amitraz was given continuously every 40 min intervals into ear vein after an initial 30 min resting period. Each value represents as mean±SEM (n = 10~12).

Effect of adrenergic and cholinergic antagonists on amitraz-induced bradycardiac responses : To know what was involved in reduction of heart rate, we used adrenergic and cholinergic antagonists; prazosin as an α_1-adrenergic antagonist, yohimbine as an α_2-adrenergic antagonist, propranolol as a β-adrenergic antagonist and atropine as a cholinergic antagonist. All of antagonists were treated at 10~15 minutes before amitraz treatment.

Pretreatment of prazosin (0.5mg/kg) did not affect significantly on the bradycardiac effect of amitraz (Fig 2). Pretreatment of yohimbine (2.0mg/kg) was showed to inhibit

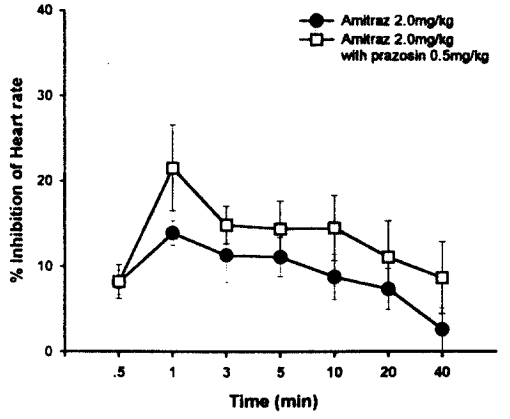

Fig 2. Effect of prazosin on the amitraz-induced bradycardia. Each value represents as mean ± SEM (n = 4).

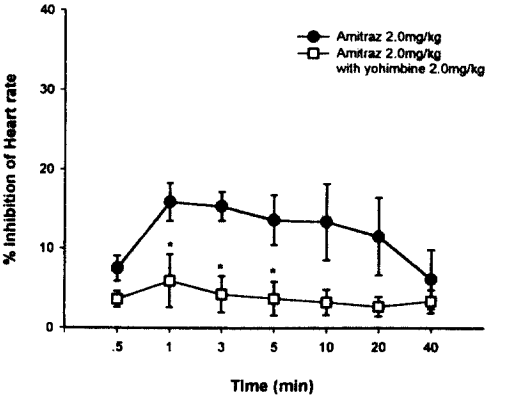

Fig 3. Effect of yohimbine on the amitraz-induced bradycardia. Each value represents as mean ± SEM (n = 6).
*p < 0.05 compared with amitraz alone.

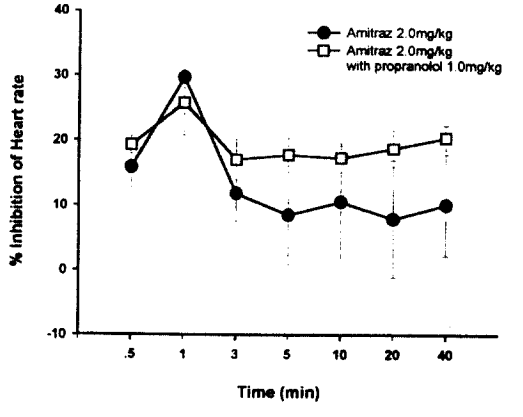

Fig 4. Effect of propranolol on the amitraz-induced bradycardia. Each value represents as mean ± SEM (n = 4).

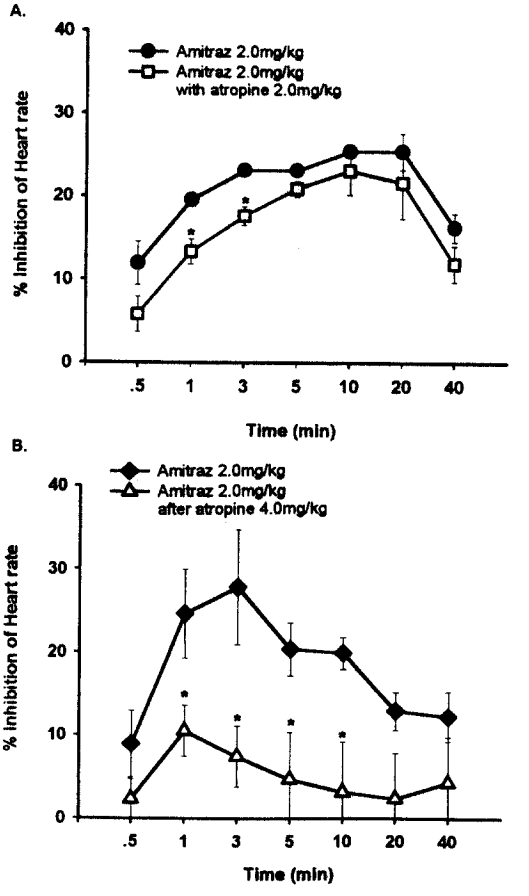

Fig 5. A. Effect of atropine 2.0mg/kg on the amitraz-induced bradycardia (n = 4).
B. Effect of atropine 4.0mg/kg on the amitraz-induced bradycardia. Each value represents as mean ± SEM (n = 5~6).
*p < 0.05 compared with amitraz alone.

the bradycardiac effect of amitraz. Especially as shown in figure 3, the inhibitory effect was more significant in 1, 3, and 5 mins ($p < 0.05$). Pretreatment of dl-propranolol (1.0mg/kg) did not influence the bradycardiac effect of amitraz (Fig 4). Pretreatment of atropine (2mg/kg and 4.0mg/kg) was showed to inhibit the bradycardiac effect of amitraz. As shown in figure 5, The effect at 4.0mg/kg was more significant than at 2.0mg/kg. As shown in the figure 6, vagotomy inhibited the bradycardiac effect of amitraz. Its effect was higher than that of atropine (2.0mg/kg) pretreated.

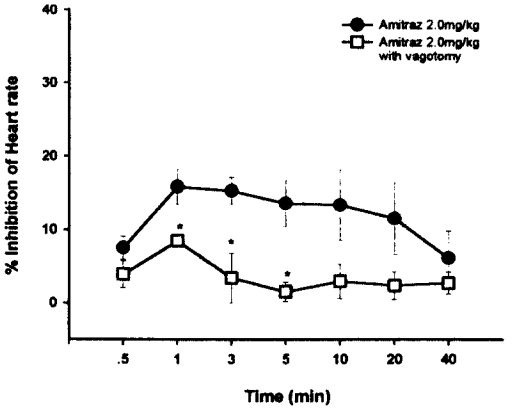

Fig 6. Effect of vagotomy on the amitraz-induced bradycardia. Each value represents as mean ± SEM (n = 4).
*p < 0.05 compared with amitraz alone.

Discussion

We treated various doses of amitraz to characterize bradycardiac effect of the drug. The present findings demonstrated that IV injection of amitraz induced bradycardiac effect in a dose-dependent manner. Seawright[11] described the toxic signs of amitraz in the dog as an initial bradycardia followed, in severely intoxicated animals, by tachycardia and hyperventilation.

To investigate the mechanisms involved in the bradycardiac effect of amitraz, we treated the following adrenergic and cholinergic antagonists before intravenous injection of amitraz; prazosin as an α_1-adrenergic antagonist, yohimbine as an α_2-adrenergic antagonist, propranolol as a β-adrenergic antagonist, and atropine as a cholinergic antagonist. And we also operated vagotomy to confirm vagal influence on the bradycardiac effect of amitraz. Amitraz-induced bradycardia was not affected by pretreatment of prazosin (0.5mg/kg) or propranolol (1.0mg/kg). But the bradycardiac effect was inhibited by pretreatment of yohimbine (2.0mg/kg).

These results suggest that bradycardiac effect of amitraz was mediated via α_2-adrenoceptors because (1) yohimbine, an α_2-adrenoceptor blocking agent, antagonized amitraz-induced bradycardiac effect and (2) prazosin, an α_1-adrenoceptor blocking agent, did not antagonize the effects of amitraz. The exact mechanisms which amitraz induced bradycardia are not known. Hsu and Kakuk[12] showed that the bradycardiac effects of amitraz in rats pretreated with α-methyl-p-tyrosine and reserpine were absent but were marked those in non-treated animals. It means that amitraz-induced bradycardia appears to be due to activation of presynaptic α_2-adrenoceptors because bradycardia was marked in nonpretreated but was absent in pretreated rats. There is a suggestion that the bradycardia may have resulted from an increased concentration of transmitter at muscarinic sites and be most likely mediated through the release of endogenous acetylcholine[13]. To examine the suggestion, we pretreated rabbits with atropine (2.0 and 4.0mg/kg) and operated vagotomy before administration of amitraz. And the bradycardiac effect was inhibited by pretreatment of atropine or operation of vagotomy.

These results suggest that amitraz may reduce heart rate due to increasing acetylcholine through activating vagal nerves and α_2-adrenoceptors which is involved in activating vagal nerves.

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