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Comparison of the arrhythmogenicity of acepromazine, xylazine and their combination in pentobarbital-anesthetized rats

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Summary

The Purpose of this study was to evaluate the effects of xylazine, acepromazine and their combination on epinephrine-induced arrhythmias during sodium pentobarbital anesthesia in male rats. Forty Sprague-Dawley male rats (240-370 g) were divided into four groups and received one of the following treatments intravenously: saline, xylazine (5 mg/kg), acepromazine (2.5 mg/kg) and xylazine-acepromazine combination (2.5 and 5 mg/kg, respectively). Rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and the arrhythmogenic dose of epinephrine (ADE) was determined by epinephrine infusion for 1 min at increasing dose rates (2.5, 5, 10, 15, 20, 30 and 60 µg/kg/min, IV) prior to, and 10 minutes after, saline or drug administration. The ADE was defined as the total dose of epinephrine that induced at least 2 ectopic ventricular depolarizations within 15 seconds during infusion or within 1 minute after the end of the infusion. Mean ADE values in the xylazine group was significantly lower than baseline values ($p<0.05$), whereas arrhythmias satisfying ADE criteria were not observed at the maximum infusion rate of 60 µg/kg/min in acepromazine or xylazine-acepromazine groups. This study showed that acepromazine alone or in combination with xylazine has a protective effect on epinephrine-induced arrhythmia in pentobarbital anesthetized rats.

Introduction

Preanesthetic medications are often used in combination with injectable anesthetics in a variety of laboratory animal species. Simultaneous administration of sedative drugs, such as alpha$_2$-adrenergic agonists and phenothiazines, provides muscle relaxation and reduces induction doses of anesthetic agents. However, these drugs may have significant cardiovascular and arrythmogenic effects which may contribute to anesthetic morbidity and mortality *(Dyson et al., 1998)*.

Results of previous reports indicate that xylazine, an alpha$_2$-adrenergic agonist, may sensitize the myocardium to epinephrine in dogs anesthetized with halothane *(Muir et al., 1975; Tranquilli et al., 1986)*, isoflurane *(Tranquilli et al., 1988)* and ketamine *(Wright et al., 1987)*; whereas, acepromazine, a phenothiazine tranquilizer, possessed a protective action against catecholamine-induced arrhythmia in dogs anesthetized with halothane *(Muir et al., 1975; Dyson & Pettifer, 1997)*. The male rat has been used as an animal model to determine the arrhythmic doses of epinephrine during halothane and isoflurane anesthesia *(Laster et al., 1990)*.

Rats are commonly used for scientific research and may be anesthetized using injectable or inhalant anesthetic agents for a variety of surgical procedures

(Flecknell, 2009); however, injectable anesthetics are commonly preferred in a laboratory setting.

Pentobarbital, as a short acting barbiturate anesthetic, is used for short surgical procedures in rats. It is rapidly absorbed following intraperitoneal administration and provide anesthesia for up to 60 min in the rat *(Flecknell, 2009)*.

The purpose of this study was to evaluate the effects of clinical doses of acepromazine, xylazine and their combination on the occurrence of epinephrine induced arrhythmia in rats under pentobarbital anesthesia.

Materials and methods

Forty Sprague-Dawley male rats (*Rattus norvegicus*) weighing 240-370 g (mean±SD 312±10 g) were used in this study. The present study was approved by the Institutional Animal Care and Use Committee. Rats were acquired from the Laboratory Animal Research Center, Shiraz University of Medical Sciences, Shiraz, Iran, and were housed in groups of four on hardwood shavings, on a 12: 12-h light-dark cycle with food and water *ad libitum*. All rats were anesthetized with 50 mg/kg intraperitoneal sodium pentobarbital. Tail veins were cannulated with two polyethylene tubes, one used for injection of sedative drugs and the other one for infusion of epinephrine. Heparinized saline was used to prevent clot formation in the tail veins. Lead II electrocardiogram was recorded continuously using Powerlab (Powerlab, AD Instrument, Australia). Heart rate and rectal temperature were monitored throughout the study. A heating lamp was used to maintain rectal temperature at 37.5-38.5° C. Hamilton syringes (500µl, Hamilton Switzerland) and automatic microinjection pumps (Stoelting CO, USA) were used for drug administration.

Rats were divided into four groups and following baseline ADE determination received one of the following treatments intravenously: saline, xylazine (5 mg/kg, Woerden-Holland), acepromazine (2.5 mg/kg, Woerden-Holland) and xylazine-acepromazine combination (2.5 and 5 mg/kg, respectively). These doses were selected based on the recommended dose of xylazine and acepromazine in rats *(Flecknell, 2009)*.

A baseline ADE was determined before drug administration (pentobarbital anesthesia alone) by epinephrine (Darou Pakhsh, Iran) infusion for 1 min at increasing dose rates (2.5, 5, 10, 15, 20, 30 and 60 µg/kg/min, IV) in each rat. The experiment was terminated at a maximum infusion rate of 60 µg/kg/min in all groups. Due to the failure to observe arrhythmia with lower doses of epinephrine in acepromazine and xylazine-acepromazine groups in the preliminary study, the starting dose was 20 µg/kg/min in these groups. Epinephrine was freshly diluted with normal saline to produce a concentration of 100 µg/mL. The ADE was defined as the total dose of epinephrine that induced at least 2 ectopic ventricular depolarizations within 15 seconds during infusion or within 1 minute after the end of the infusion. If arrhythmia was not observed at one rate, the next higher infusion rate was begun 10 minutes later. The total dose (µg/kg) of epinephrine was calculated as a function of infusion rate and time to arrhythmia formation.

Statistical analysis: All data are presented as mean±SD. Paired t-test was performed to determine differences between baseline and treatment ADE values in each group. A one-way ANOVA followed by Duncan's test was used to compare heart rate and rectal temperature. Statistical analysis was undertaken using SPSS Version 10 for Windows (SPSS, MicroMaster, Richboro, PA, USA) and $p<0.05$ was considered significant.

Results

No significant differences were found in the baseline ADE between treatment groups. The ADE was not significantly different from the baseline value following the administration of saline (Table 1). Xylazine administration significantly reduced the ADE from 11.6±4.1 µg/kg to 8.4±4.3 µg/kg ($P<0.05$). Arrhythmias satisfying the ADE criteria were not observed at the maximum infusion rate of 60 µg/kg/min in rats receiving acepromazine alone or in combination with xylazine. Heart rate significantly decreased following xylazine (349±34 vs. 288±33 beats/min) and acepromazine-xylazine (356±31 vs. 314±49 beats/min) administration but no changes in saline and acepromazine groups were observed. There were no significant differences in rectal temperature over time within treatment groups.

Discussion

Determination of the arrhythmogenic dose of epinephrine (ADE) has been used to investigate the abil-

Table 1. The arrhythmogenic dose of epinephrine (ADE) following the administration of saline, xylazine, acepromazine or xylazine/acepromazine in pentobarbital-anesthetized male rats.

Treatment groups	Baseline ADE (μg/kg) (Pentobarbital alone)	Treatment ADE (μg/kg)
Saline	10.2±2.5	10.2±2.7
Xylazine	11.6±4.1	8.4±4.3*
Acepromazine	11.0±3.5	> 60*‡
Xylazine/ Acepromazine	10.7±3.6	> 60*‡

Data presented as mean±SD (n = 10)
*Significant differences from baseline ADE (P<0.05).
‡ No ADE at maximal infusion rate (60 μg/kg)

ity of several anesthetic and sedative drugs to alter myocardial sensitivity to catecholamines *(Lemke & Tranquilli, 1994)*. Several factors may influence alteration in arrhythmogenicity, including dosage and route of administration of drugs, animal species and sex, and the method of epinephrine administration (bolus injection vs. infusion) *(Lemke & Tranquilli, 1994)*.

A sex difference in susceptibility to epinephrine-induced arrhythmias has been reported in Sprague-Dawley rats *(Laster et al., 1990)*. Male rats were more susceptible than females to the arrhythmic effects of epinephrine. In the present study, only male rats were used to eliminate the effects of sex on myocardial susceptibility to epinephrine.

In the present study, the ADE values during pentobarbital anesthesia were higher than previously reported values for rats anesthetized with halothane (2.4±1.0 μg/kg) *(Laster et al., 1990)*; but lower than values reported for isoflurane anesthetized rats (28.4±24.0 μg/kg). In another study using bolus injection of epinephrine, the arrhythmogenic threshold of epinephrine during pentobarbital anesthesia (39.0±3.9 μg/kg) was higher compared to halothane (1.7±3.2 μg/kg) or isoflurane (11.1±0.6 μg/kg) anesthesia in rats *(Takada et al., 1993)*. However, in these studies, epinephrine has been administered as intravenous bolus injections. Due to the difference in method of epinephrine administration, it is difficult to compare the results of these studies with the present study. The results of this study indicate that repeated administration of intravenous epinephrine to pentobarbital anesthetized rats does not alter the arrhythmogenic dose of epinephrine.

Pentobarbital, as a short-acting oxybarbiturate, does not appear to alter cardiac arrhythmogenicity in dogs and rats *(Sumikawa et al., 1983; Takada et al., 1993)*. It has been reported that the ADE during pentobarbital anesthesia in rats is 3.5 and 23 times greater than that during isoflurane or halothane anesthesia, respectively *(Takada et al., 1993)*. However, thiobarbiturates, thiopental and thiamylal, sensitize the heart to catecholamine-induced arrhythmias in halothane-anesthetized dogs *(Atlee & Malkinson 1982; Atlee & Roberts 1986; Bednarski et al., 1985; Hayashi et al., 1989)*.

Xylazine and acepromazine have been used in combination with other injectable agents for the production of general anesthesia in rats *(Flecknell, 2009)*. Xylazine induces vasoconstriction, transient hypertension, reflex bradycardia and prolonged hypotension. Sedative doses of acepromazine cause vascular α_1-receptor blockade and subsequent hypotension *(Lemke, 2007)*. Acepromazine has antiarrhythmic effects, while xylazine can cause cardiac arrhythmias. A combination of acepromazine and xylazine has been used in horses *(Muir et al., 1979; Nilsfors et al., 1988; Hubbell et al., 1999) dogs (Cronin et al., 1983)* and rats *(Flecknell, 2009)*.

The findings concerning the effects of xylazine and acepromazine on the ADE are in agreement with previous studies using halothane or isoflurane anesthetized dogs *(Muir et al., 1975; Tranquilli et al., 1986; Tranquilli et al., 1988)*. In addition, acepromazine in combination with xylazine not only prevented xylazine-induced arrhythmia, it also provided a protective effect to the same extent as acepromazine alone.

The mechanism of ADE reduction following xylazine administration is not completely understood. Xylazine, as a mixed α_1- and α_2-adrenoreceptor agonist, has stimulatory effects at both peripheral and central receptors. Increased arrhythmogenicity after xylazine administration in rats might be attributable to activation of α_1-adrenoreceptors, because α_2-adrenoreceptors are absent on myocardial cells and stimulation of central α_2-adrenoreceptors reduces arrhythmogenicity by decreasing sympathetic outflow *(Hayashi et al., 1991)*. Administration of dexmedetomidine, a specific α_2-adrenoreceptor agonist (α_2 to α_1 specificity of 1,600 to 1) has showed antiarrhythmic actions in halothane anesthetized dogs

(Hayashi et al., 1991). The antiarrhythmic effects of dexmedetomidine were blocked by atipamezole, a specific α_2-adrenoreceptor antagonist. Interestingly, yohimbine, an α_2-adrenoreceptor antagonist with α_2 to α_1 specificity of 606 to 1, reduces arrhythmogenicity of xylazine in halothane-anesthetized dogs *(Tranquilli et al., 1988)*, indicating that partial blockade of α_1, in addition to α_2-adrenoreceptors, decreases the potential for ventricular arrhythmias.

In the present study, blockade of cardiovascular α_1-adrenoreceptors with acepromazine could be the most likely mechanism responsible for prevention of epinephrine-induced arrhythmias in rats given acepromazine alone or in combination with xylazine *(Muir et al., 1975; Lemke & Tranquilli 1994; Dyson & Pettifer 1996)*. The vasodilatory properties, with resultant hypotension, may also play a role in antiarrhythmic activities of acepromazine.

In conclusion, xylazine reduced the ADE in pentobarbital-anesthetized rats, while acepromazine was effective in preventing epinephrine-induced arrhythmias even in the presence of xylazine in rats anesthetized with pentobarbital. Protective effects of acepromazine may reduce the chance of ventricular arrhythmias related to anesthesia in rats.

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