The Parasympatholytic Effects of Atropine Sulfate and Glycopyrrolate in Rats and Rabbits

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ABSTRACT

Nine groups of rats (n=5 per group) received an intramuscular (IM) injection of one of the following drugs or drug combinations: saline, atropine (0.05 mg/kg), glycopyrrolate (0.5 mg/kg), ketamine:xylazine (85:15 mg/kg), ketamine:detomidine (60:10 mg/kg), atropine:ketamine:xylazine (0.05: 85:15 mg/kg), glycopyrrolate: ketamine:xylazine (0.5:85:15 mg/kg), atropine:ketamine:detomidine (0.05: 60:10 mg/kg) or glycopyrrolate: ketamine:detomidine (0.5:60:10). Similarly six groups of rabbits (n=5) received an IM injection of either saline, atropine (0.2 mg/kg), atropine (2 mg/kg), glycopyrrolate (0.1 mg/kg), ketamine:xylazine (35:10 mg/kg) or glycopyrrolate:ketamine:xylazine (0.1:35:10 mg/kg).

In rats, atropine sulfate (0.05 mg/kg) and glycopyrrolate (0.5 mg/kg) produced an increase in heart rate for 30 and 240 min, respectively. In rabbits atropine sulfate at either 0.2 or 2.0 mg/kg did not induce a significant increase in heart rate, but glycopyrrolate (0.1 mg/kg) elevated the heart rate above saline treated animals for over 50 min. Both atropine and glycopyrrolate provided protection against a decrease in heart rate in rats anesthetized with ketamine: xylazine (85:15 mg/kg) or ketamine: detomidine (60:10 mg/kg); however, glycopyrrolate was significantly more effective in maintaining the heart rate within the normal range. Glycopyrrolate also prevented a decrease in heart rate in rabbits anesthetized with ketamine:xylazine (35:5 mg/kg). Neither glycopyrrolate nor atropine influenced respiration rate, core body temperature or systolic blood pressure when used alone or when combined with the injectable anesthetic. Glycopyrrolate is an effective anticholinergic agent in rabbits and rodents and more useful as a preanesthetic agent than atropine sulfate in these animals.

RÉSUMÉ

Neufs groupes de rats (n=5) ont reçu une injection intramusculaire (IM) de l'un des médicaments ou de l'un des mélanges médicamenteux suivants: saline, atropine (0,05 mg/kg), glycopyrrolate (0,5 mg/kg), kétamine: xylazine (85:15 mg/kg), kétamine: détomidine (60:10 mg/kg), atropine: kétamine:xylazine (0,05:85:15 mg/kg), glycopyrrolate:kétamine:xylazine (0,5:85:15 mg/kg), atropine:kétamine: détomidine (0,05:60:10 mg/kg) ou glycopyrrolate:kétamine:détomidine (0,5: 60:10 mg/kg). Six groupes de lapins (n=5) ont reçu une injection intramusculaire de l'un des traitements suivants: saline, atropine, (0,2 mg/kg), atropine (2 mg/kg) glycopyrrolate (0,1 mg/kg), kétamine:xylazine (35:10 mg/kg) ou glycopyrrolate:kétamine: xylazine (0,1:35:10 mg/kg).

Chez les rats, le sulfate d'atropine (0,05 mg/kg) et le glycopyrrolate (0,5 mg/kg) ont induit une augmentation de la fréquence cardiaque pendant 30 et 240 min respectivement. Chez les lapins, le sulfate d'atropine, que ce soit à 0,2 ou 2,0 mg/kg, n'a pas provoqué d'augmentation significative de la fréquence cardiaque, mais le glycopyrrolate (0,1 mg/kg) entraîna une augmentation de la fréquence cardiaque par rapport aux animaux ayant reçus de la saline, et cela pour 50 minutes. L'atropine et le glycopyrrolate ont assuré une protection contre une diminution de la fréquence cardiaque chez les rats anesthésiés avec les combinaisons kétamine:xylazine (85:15 mg/kg) ou kétamine:détomidine (60:10 mg/kg), le glycopyrrolate étant plus efficace, et cela de façon significative, à maintenir la fréquence cardiaque dans les limites normales. Le glycopyrrolate a aussi empêché une diminution de la fréquence cardiaque chez les lapins anesthésiés avec la combinaison kétamine:xylazine (35:5 mg/kg). L'atropine et le glycopyrrolate n'ont pas eu d'influence sur la fréquence respiratoire, la température corporelle centrale et la pression artérielle systolique, que ce soit lors de leur utilisation respective individuelle ou combinée avec les agents anesthésiques. Le glycopyrrolate est un agent anticholinergique efficace chez les lapins et les rongeurs et est plus utile comme agent préanesthésique que le sulfate d'atropine chez ces espèces. (*Traduit par Dre Sophie Cuvelliez*)

INTRODUCTION

The use of anticholinergic agents are controversial and their efficacy varies greatly with species (1–6). They provide beneficial effects by preventing bradycardia and accumulation of salivary secretions that can occur during anesthesia and surgical manipulations. They are often recommended in studies using laboratory animals although there is limited information on efficacy in these species (1–6). Prevention of bradycardia is particularly important during lengthy surgical and experimental procedures involving these laboratory animals. *Alpha*-2-adrenergic agents, such as xylazine, which are a common component of injectable anesthetic combinations in laboratory animals, produce severe depression of heart rate in rodents and rabbits (3–6). Endotracheal intubation or the surgical manipulation of

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TABLE I. Experimental groups

Group	Species	Preanesthetic	Dosage (mg/kg)	Anesthetic	Dosage (mg/kg)
1	rat	saline	N/A[a]	none	N/A
2	rat	atropine	0.05	none	N/A
3	rat	glycopyrrolate	0.5	none	N/A
4	rat	none	N/A	K[b]:X[c]	85:15
5	rat	none	N/A	K:D[d]	60:10
6	rat	atropine	0.05	K:X	85:15
7	rat	glycopyrrolate	0.5	K:X	85:15
8	rat	atropine	0.05	K:D	60:10
9	rat	glycopyrrolate	0.5	K:D	60:10
10	rabbit	saline	N/A	none	N/A
11	rabbit	atropine	0.2	none	N/A
12	rabbit	atropine	2.0	none	N/A
13	rabbit	glycopyrrolate	0.1	none	N/A
14	rabbit	none	N/A	K:X	35:5
15	rabbit	glycopyrrolate	0.1	K:X	35:5

[a] N/A = not applicable
[b] K = ketamine hydrochloride
[c] X = xylazine hydrochloride
[d] D = detomidine hydrochloride

viscera, which are common procedures performed in research, can lead to vagal stimulation often resulting in severe bradycardia (7). The use of anticholinergic agents for premedication may prevent or eliminate the bradycardia caused by *alpha*-2 adrenergic agents or vagal stimulation. The accumulation of bronchial and salivary secretions in the respiratory tract is often an anesthetic complication associated with the use of many anesthetic agents in laboratory animals and may be prevented by the use of anticholinergic agents as a premedication (1,7).

Atropine is a plant alkaloid that has been used extensively in most animal species as an anticholinergic agent (1). Although atropine is recommended as a preanesthetic agent in laboratory rodents and rabbits the efficacy of this drug is controversial (2,5,6,7). Rodents and rabbits have a high metabolic rate and rapidly eliminate atropine from the body (2,6,7). Some rabbits also may have high levels of serum atropine esterase activity that rapidly hydrolyses atropine (8,9). The enzyme is coded for by a semidominant gene and reportedly accounts for the rabbit's ability to thrive on a diet of belladonna leaves (8,9). Glycopyrrolate is a synthetic anticholinergic agent. It has a longer duration of action than atropine (1, 10) and may not be as susceptible to hydrolytic degradation by serum atropine esterase.

This study was conducted to evaluate the influence of atropine and glycopyrrolate on heart rate, systolic blood pressure, respiration rate and core body temperature as well as the efficacy of these anticholinergic agents in preventing bradycardia and sialorrhea in animals anesthetized with an injectable anesthetic combination.

MATERIALS AND METHODS

Male Sprague-Dawley rats (Crl:CD (SD)BR, Charles River Inc. St-Constant, Quebec) weighing 180-220 g and female New Zealand white rabbits (Vandermeer, Sherwood Park, Alberta) weighing 2.5–3.0 kg were used in this study. Using the *in vivo* screen test (11) 20% of rabbits from this source had a high level of atropine esterase activity. Rabbits positive for atropine esterase by the screen test were not used in the study. Rats were housed in groups of three to four in polycarbonate solid bottom cages with corn cob bedding (Bed-O-Cobs, The Andersons, Maymee, Ohio). Rabbits were housed individually in stainless steel cages. Rats and rabbits were conventionally maintained in rooms at 20 ± 2°C, 40 ± 5% relative humidity, 18 air changes per hour and 12 hours daily illumination. Tap water and commercial rodent chow (Rodent blox, Wayne Pet Food Division, Chicago, Illinois) or commercial rabbit pellets (United Feeds, Calgary, Alberta) were provided *ad libitum*. The study was reviewed by the University of Calgary Animal Care Committee and was conducted in accordance with the guidelines of the Canadian Council on Animal Care.

Animals were randomly assigned to one of the 15 test groups consisting of five animals in each group (Table I). At the end of the study animals were assigned to another unrelated study rather than being euthanized.

Atropine sulfate (Atro-Sa, Rafter 8 Products, Calgary, Alberta) and glycopyrrolate (Robinul, Ayerst Laboratories, St. Laurent, Quebec) were injected alone or in combination with the anesthetic agents (Table I) into the caudal thigh muscle. The amount of atropine sulfate injected represented a published dosage for rats of 0.05 mg/kg (5–7). Two rabbit dosages (0.2 and 2.0 mg/kg) were selected because of the wide range of published dosages of atropine sulfate for rabbits and the reported resistance to atropine sulfate of rabbits (2,5,6,7). The dosage of glycopyrrolate for rabbits was selected based on that published for dogs and cats (12) while rats received five times this dosage because of their higher metabolic activity (13).

Ketamine hydrochloride (Rogarsetic, rogar/STB, London, Ontario) was combined with xylazine (Rompun, Bayvet Division Chemagro Ltd, Etobicoke, Ontario) or detomidine (Dormosedan, SmithKline Beecham, Animal Health Products, Mississauga, Ontario) in one syringe to provide the injectable anesthetic combinations (14–16). Injectable anesthetic combinations were also given by intramuscular (IM) injection. In groups in which parasympatholytic agents were administered with the anesthetic agents, all agents were mixed in a single syringe and injected IM to reduce the stress associated with multiple injections. Control animals received sterile 0.9% saline by IM injection.

Heart rate, systolic blood pressure, respiratory rate, and core body temperature were monitored prior to and following injections in each animal (Times 0, 5, 15, 30, 60, 90, 120, 150, 180, 210, 240, 270 and 300 min for rats and Times 0, 5, 15, 30, 45, 60, 90, 120 and 150 min for rabbits). Heart rates and systolic blood pressures were determined using a Harvard Indirect Blood Pressure System (Ealing Scientific, Ltd, St. Laurent, Quebec). This procedure has been shown to be accurate, reproducible and values correlate well with direct methods for both conscious and anesthetized animals (17). It was necessary to clip the hair from the tails of rabbits in order for the photoelectric cell to detect pulsations in the caudal artery. Respiratory rates were determined by visually counting thoracic

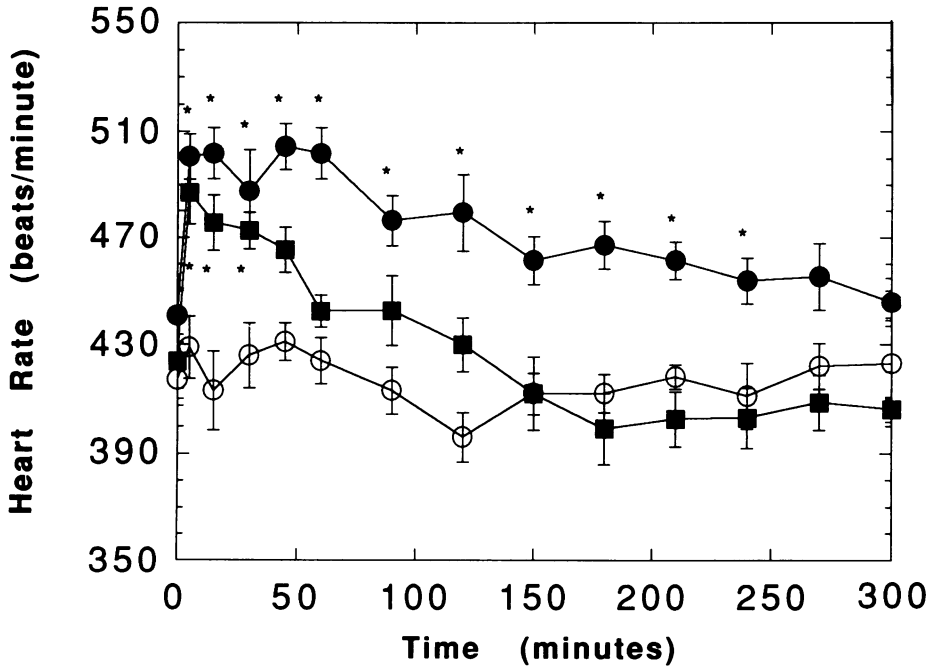

Fig. 1. Heart rate of rats injected with 0.5 mg/kg glycopyrrolate (•), 0.05 mg/kg atropine (▪) or saline (○). Values represent mean ± SE. (* = significantly different from saline treated group, p<0.05).

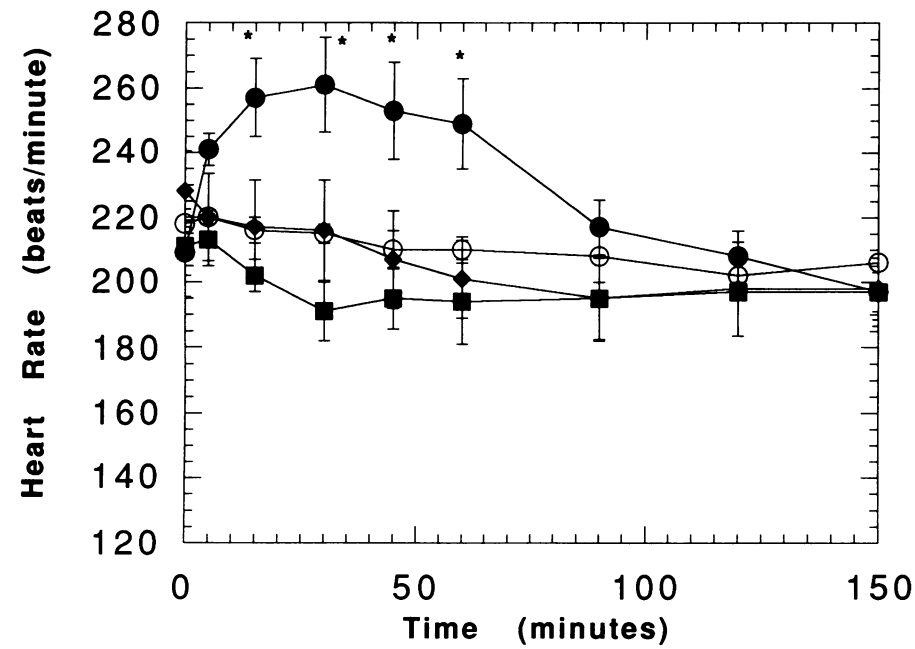

Fig. 2. Heart rate of rabbits injected with 0.1 mg/kg glycopyrrolate (•), 0.2 mg/kg atropine (♦), 2.0 mg/kg atropine (▪) or saline (○). Values represent mean ± SE. (* = significantly different from saline treated group, p<0.05).

movements. Core body temperatures were measured by using an electronic digital thermometer (Ealing Scientific Ltd) with the probe inserted approximately 2.5 and 4.5 cm into the rectum for rats and rabbits respectively.

Analysis of variance and multiple comparison of means (Tukey test) was used to determine significance among treatment groups ($p < 0.05$). Statistical analysis was performed employing SuperANOVA computer software (Abacus Concepts, Berkeley, California).

RESULTS

There were no significant differences in core body temperature, respiration rate and systolic blood pressure in rats or in rabbits receiving saline, atropine, or glycopyrrolate alone (data not shown).

Rats receiving either atropine or glycopyrrolate had significantly elevated heart rates within 5 min of the injection (Fig. 1). The heart rate was significantly increased above saline treated animals for 30 min after injection with atropine and 240 minutes in glycopyrrolate treated animals.

In rabbits glycopyrrolate produced a significantly elevated heart rate that lasted for over 60 min while atropine at 0.2 and 2 mg/kg did not elicit an increase in heart rate (Fig. 2). Salivary secretions were only reduced in glycopyrrolate treated rabbits. Since atropine appeared to have minimal vagolytic effects in rabbits it was not used in combination with ketamine:xylazine.

The heart rates of rats receiving ketamine:xylazine were significantly depressed for over 300 min compared to the saline treated animals (Figs. 3 and 4). The heart rate in ketamine:xylazine:atropine rats was significantly reduced for 60 min while significantly reduced for 15 min in rats receiving ketamine:xylazine:glycopyrrolate (Fig. 3). Rats receiving ketamine:detomidine:glycopyrrolate had heart rates that were similar to the control animals (saline) for 150 min after the injection (Fig. 4). The heart rates of rats injected with ketamine:xylazine:atropine were significantly depressed compared to the saline group for over 300 min. Glycopyrrolate was significantly more effective than atropine in reducing the duration of heart rate depression in rats anesthetized with either ketamine:xylazine and ketamine:detomidine. In rats, both ketamine:xylazine and ketamine:detomidine had no influence on respiration rate, caused a transient increase in blood pressure and decreased core body temperature as reported previously (16). These data are not shown here. Atropine and glycopyrrolate had no influence on these physiological parameters in anesthetized animals.

As described previously, rabbits receiving ketamine:xylazine did not progress to a surgical plane of anesthesia and were only heavily sedated (19). Ketamine:xylazine did not influence respiration rate, core body temperature, or systolic blood pressure in these animals (data not shown). The heart rate was significantly decreased 15 min after injection with the ketamine:xylazine

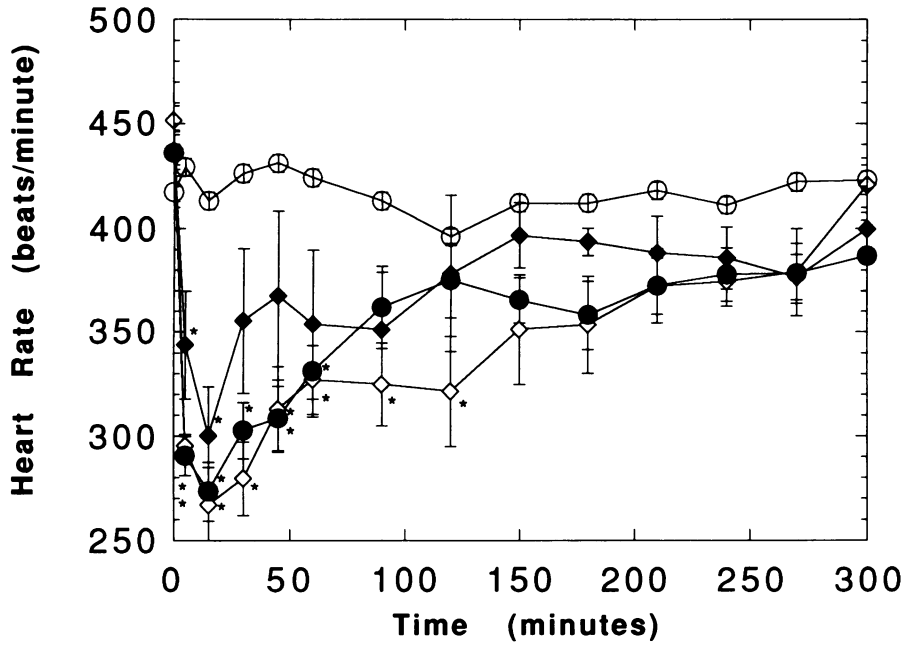

Fig. 3. Heart rate of rats injected with 85:15 mg/kg ketamine: xylazine (◦), 85:15:0.5 mg/kg ketamine:xylazine:glycopyrrolate (•), 85:15:0.05 mg/kg ketamine:xylazine:atropine (•) or saline (◦). Values represent mean ± SE. (* = significantly different from saline treated group, p<0.05).

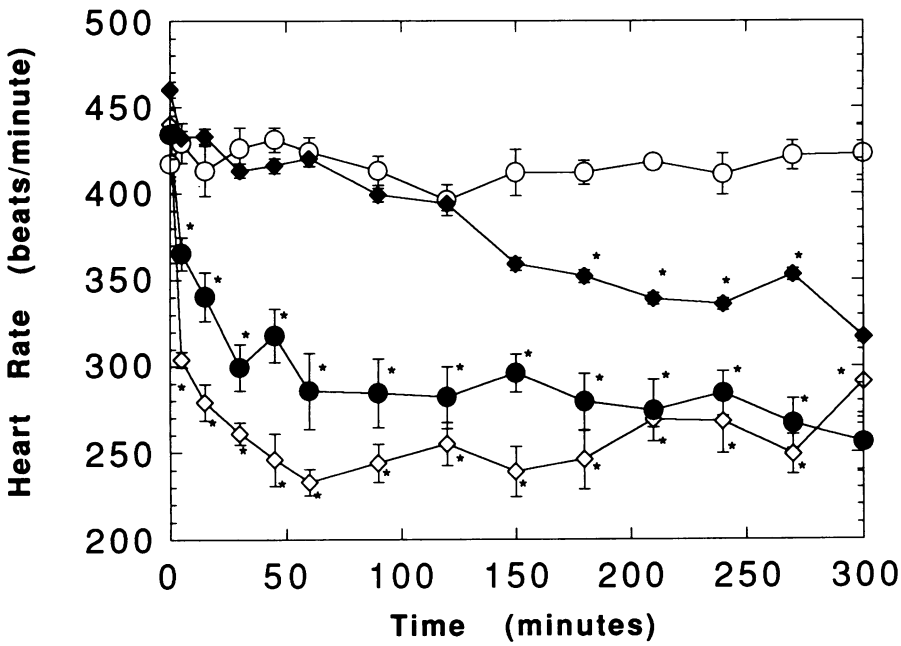

Fig. 4. Heart rate of rats injected with 60:10 mg/kg ketamine: detomidine (◦), 60:10:0.5 mg/kg ketamine:detomidine:glycopyrolate (•), 60:10:0.05 mg/kg ketamine:detomidine:atropine (•) or saline (◦). Values represent mean ± SE. (* = significantly different from saline treated group, p<0.05)

combination and continued to include the 120 min observation (Fig. 5). When glycopyrrolate was mixed with this anesthetic combination the heart rate remained at the preinjection rate and did not differ from the control animals (Fig. 5). There were no significant differences in respiration rate, core body temperature, or systolic blood pressure between groups receiving ketamine: xylazine and ketamine:xylazine:glycopyrrolate (data not shown).

DISCUSSION

In anesthetized dogs and cats, both atropine sulfate and glycopyrrolate are effective in inhibiting sialorrhea and intestinal peristalsis as well as controlling bradycardia (12,19,20); however, in these species, glycopyrrolate has been shown to be more potent and have more long-lasting effects than atropine (12,19). Glycopyrrolate also differs from atropine in that it does not significantly cross the placental and blood-brain barriers (1).

Many text books recommend the use of atropine as a preanesthetic medication for rats and rabbits; however, there is considerable variation in reported dosages and information on efficacy is not provided (2,5,6,7). This study demonstrated that atropine causes a brief period of elevated heart rate in unanesthetized rats and only weakly controls the heart rate depression in ketamine:xylazine or ketamine:detomidine anesthetized rats. Conversely, glycopyrrolate was more effective in preventing the heart rate depression in anesthetized rats. This may be attributed to a reduced rate of hydrolytic degradation and a higher anticholinergic activity in rats.

Atropine sulfate in rabbits did not elevate the heart rate at either dosage used. These rabbits were outbred stock from a single supplier with a high level of serum atropine esterase in 20% of the animals; however, the assay system employed to detect serum atropine esterase levels may only detect animals with very elevated levels of the enzyme. Despite only animals without a detectable atropine esterase activity (according to a screening test) being used in this study, atropine still had no detectable vagolytic effect on these animals. Glycopyrrolate was effective in preventing the heart rate depression in rabbits receiving ketamine:xylazine. This is consistent with the rat data in this study and previous reports that glycopyrrolate in dogs and cats was more potent with longer lasting anticholinergic effects (12,18,19).

In this study core body temperature was not controlled and supplemental oxygen was not provided. Both of these variables may have an influence on the activity and metabolism of both atropine and glycopyrrolate (21). In this study body temperature was not influenced

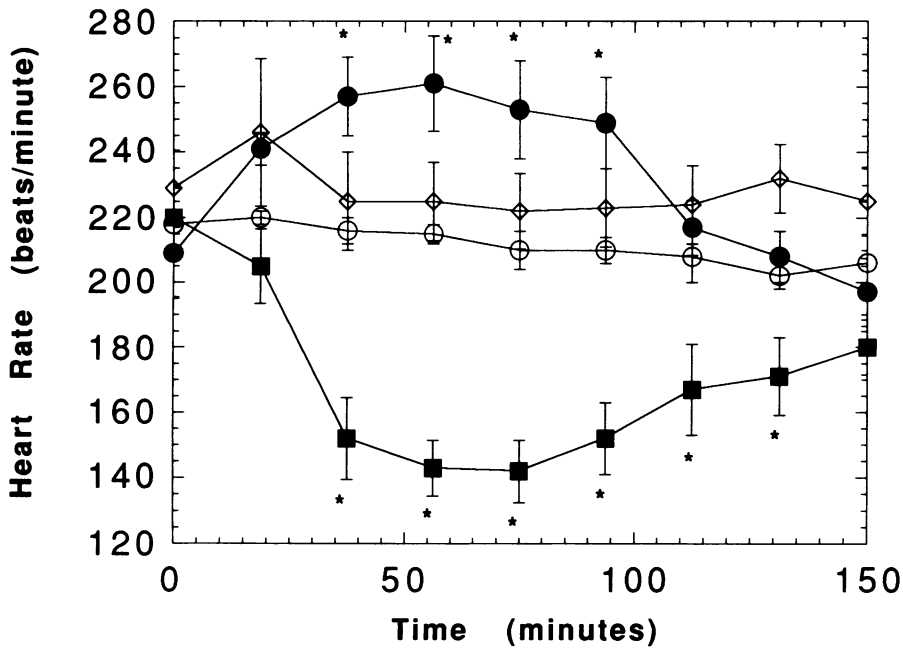

Fig. 5.Heart rate of rabbits injected with 35:5 mg/kg ketamine:xylazine (▪), 35:5:0.1 mg/kg ketamine:xylazine:glycopyrrolate (◦), 0.1 mg/kg glycopyrrolate (•), or saline (○). Values represent mean ± SE. (* = significantly different from saline treated group, p<0.05).

in rabbits and rats receiving only saline, atropine or glycopyrrolate. Core body temperature was depressed in anesthetized rats which may have influenced the results in those groups. Ketamine: xylazine have been shown to influence body temperature and blood gases in laboratory animals (23). The rationale for not providing supplemental oxygen or heat is they are not routinely used during rat and rabbit anesthesia/sedation, thus the information obtained can be applied to the research laboratory.

This study suggests there are no beneficial effects to the heart rate by using atropine sulfate as a preanesthetic medication in rabbits. Conversely, glycopyrrolate was an effective anticholinergic agent in rats and rabbits controlling heart rate depression. Glycopyrrolate should prove to be a useful preanesthetic medication in these species.

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