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Mechanism of Tachycardia Caused by Intracarotid PGE_2 in Conscious Ewes (42188)

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Abstract. Conscious adult ewes prepared with nonocclusive indwelling vascular catheters were used to determine the mechanism by which heart rate increases during central administration of prostaglandin E_2 (PGE_2). Heart rate increased 14 bpm during steady-state intracarotid infusion of PGE_2, 10 ng/kg/min ($P < 0.05$). Intravenous atropine methyl bromide, 1 mg/kg, increased heart rate 26 bpm ($P < 0.05$) 5 min after injection. Heart rate remained elevated 30 min after injection. The heart rate response to PGE_2 plus atropine was greater than the heart rate response to either atropine or PGE_2 alone ($P < 0.05$). Propranolol, 1 mg/kg bolus plus intravenous infusion, 0.025 mg/kg/min, did not change resting heart rate. Propranolol attenuated but did not abolish the increase in heart rate caused by intracarotid PGE_2. Although heart rate increased in response to PGE_2 after administration of either propranolol or atropine alone, the combination of propranolol and atropine prevented any further increase in heart rate during subsequent PGE_2 infusion. The increase in heart rate when all three drugs were given together was not different from the increase observed during atropine alone. Thus, both β-adrenergic activation and muscarinic deactivation contribute to the PGE_2-induced tachycardia.

Prostaglandin E_2 (PGE_2) is synthesized by brain vascular and neural tissue (1, 2) and may be involved in central arterial pressure regulation (3, 4). Although PGE_2 is a potent peripheral vasodilator (5), it increases blood pressure and heart rate when infused into a carotid artery (10 ng/kg/min) in conscious sheep (6, 7). These effects are not observed in anesthetized animals (7, 8), and are believed to be caused by action of PGE_2 in the brain, since intracarotid (IC) PGE_2 does not act at the carotid sinus (6, 9) and since this amount of PGE_2 is metabolized completely by the lungs and does not enter the peripheral circulation (5, 7). The central pressor effect of PGE_2 also is observed when PGE_2 is administered into the cerebral ventricles (IVT) (10–15), but in sheep IVT PGE_2 has less potent pressor action than IC PGE_2 and IVT PGE_2 does not alter heart rate (10).

Previous studies have focused on the mechanism of the pressor response to central PGE_2 (7, 10–15). In sheep, the increase in blood pressure during IC PGE_2 infusion is caused by increased total peripheral resistance (6, 7) and can be prevented by phentolamine (7). The present study was performed to determine the mechanism of the increase in heart rate caused by IC PGE_2 infusion in conscious sheep.

Materials and Methods. Eight mature ewes, ranging in weight from 50 to 80 kg, were used for the PGE_2 infusions and muscarinic receptor blockade experiments. Seven of these same sheep also were used for the β-adrenoceptor blockade experiments and the combined blockade experiments. The sheep were housed individually in the laboratory and they were allowed to acclimate to their surroundings and laboratory personnel. This ensured that the sheep were calm during the experiments.

Experiments were repeated in each sheep a minimum of twice. Each sheep was given at least 1 day to recover after PGE_2 or β-adrenoceptor blockade experiments, and at least 3 days after muscarinic receptor blockade experiments. The values obtained from repeated experiments in any one sheep were averaged to produce mean values characteristic of that sheep. Statistical analysis was performed on the data per sheep rather than per replicate.

Surgical preparation. The sheep were anesthetized with intravenous sodium thiamylal, intubated, and maintained with halothane and oxygen. They were placed on their backs and their necks were prepared for aseptic surgery. Both carotid arteries were exposed without disturbing the vagus nerves, and nonocclusive polyvinyl catheters were implanted (16). One external jugular vein also was catheterized (16). The catheters were tunneled subcutaneously to a spot near the shoulder, brought through the skin individually, and tied to a

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loop of polyvinyl tubing. Immediately after surgery each sheep was given 5×10^4 units/kg procaine penicillin G intramuscularly and 1.92 g trimethoprim-sulfa (Tribrissin; Burroughs Wellcome Co.) orally, and they were maintained on the trimethoprim-sulfa for an additional 3 days. The sheep were given 2 weeks to recover and to become accustomed to handling. The catheters were filled daily with 1:1000 sodium heparin.

PGE_2 infusions. With a sheep standing quietly in its cage, mean and pulsatile arterial blood pressure were recorded continuously from a carotid artery catheter onto a Gould–Brush chart recorder using Gould–Statham pressure transducers zeroed at the level of the heart. Data also were recorded onto magnetic tape for storage and playback using a Hewlett–Packard FM tape recorder. Heparinized saline was infused into a carotid artery (IC) at a rate of 0.3 ml/min. Control blood pressure and heart rate were obtained in this manner and then the heparinized saline was discontinued and PGE_2, 10 ng/kg/min, was infused IC at the same rate, 0.3 ml/min. (PGE_2 was made up as a stock solution of 1 mg/ml in ethanol and stored at −20°C. Just prior to infusion the proper amount was diluted in physiological saline.) PGE_2 infusion was continued for 35 min. Blood pressure and heart rate data were averaged over a 2-min period during control and at the beginning of each 5 min interval during PGE_2 infusion. Steady-state PGE_2 values were obtained 25 min into the infusions. The average number of replicates per sheep was six. There was no tachyphylaxis to PGE_2 infusion in any sheep.

Muscarinic receptor blockade. Three series of experiments were performed with atropine. Timed control experiments were performed by giving atropine by itself. In a second series of experiments atropine was given 5 min before the start of IC PGE_2 infusion. Finally, atropine was given 22 min after the start of IC PGE_2 infusion. Muscarinic receptor blockade was produced with atropine methyl bromide (Sigma), 1 mg/kg iv. Atropine methyl bromide was used in these experiments because it does not cross the blood–brain barrier (17). Effectiveness of the blockade was tested with acetylcholine (ACH), and will be described in the results.

β-Adrenoceptor blockade. Three series of experiments were performed with propranolol. In one series of experiments, propranolol was given by itself to serve as a time control. In a second series of experiments, propranolol was given 10 min before the start of IC PGE_2 infusion. In a third series of experiments, propranolol was given 17 min after the start of PGE_2 infusion. β-Adrenoceptor blockade was performed in seven of the same sheep used above with *d,l* propranolol (Sigma), 1 mg/kg + 0.025 mg/kg/min iv. The additional infusion of propranolol was necessary to maintain an effective level of blockade for the duration of the experiment. Effectiveness of the blockade was tested with isoproterenol, and will be described in the results.

Combined blockade. In a final series of experiments, IC PGE_2 was infused in the presence of both β-adrenoceptor and muscarinic receptor blockade. These experiments were performed in the same seven sheep used in the β-adrenoceptor blockade experiments. The same doses of blocking agents and challenging agents were used. To test the ability of the combined blockade to prevent the increases in heart rate during IC PGE_2 infusion, propranolol was started at time zero and atropine was given 5 min later. Ten minutes after the initial propranolol bolus, the IC PGE_2 infusion was started. Combined blockade also was performed in the reverse order: PGE_2 was started at time zero, propranolol was given 17 min after the start of PGE_2 infusion, and atropine was given 22 min after the start of infusion.

Statistical analysis. For each variable (blood pressure and heart rate), control and experimental values corresponding to 25 min of PGE_2 infusion were used. Values from replicates of experiments were averaged for each sheep and these averaged data were analyzed by blocked analysis of variance (ANOVA). Student–Newman–Keuls test for multiple comparisons was used to determine whether there were significant differences in control values of blood pressure or heart rate among experiments, or in the blood pressure or heart rate responses to PGE_2 alone, the blocking agent(s) alone, or PGE_2 plus blocking agent(s). Values of $P < 0.05$ were considered to indicate significant differences.

Results. Control values for blood pressure and heart rate for each individual sheep did not vary from day to day, and the average control values for blood pressure and heart rate for all sheep were not significantly differ-

TABLE I. MUSCARINIC RECEPTOR BLOCKADE WITH ATROPINE METHYL BROMIDE (1 mg/kg iv) ALONE, DURING, OR BEFORE IC PGE_2 INFUSION (10 ng/kg/min) IN EIGHT CONSCIOUS SHEEP

Experimental protocol	Blood pressure (mm Hg)			Heart rate (bpm)		
	Control	Experimental	SED[a]	Control	Experimental	SED
PGE_2 (25 min)	83	105*,**	(1.51)	73	87*,**	(2.09)
Atropine (5 min)	83	91*,***	(2.93)	76	102*,***	(4.18)
(30 min)	84	88*,***	(1.41)	76	100*,***	(4.83)
PGE_2 (25 min) + atropine (5 min)	82	102*,**	(1.48)	78	116*,**,***	(5.50)
Atropine (30 min) +PGE_2 (25 min)	83	105*,**	(1.42)	79	113*,**,***	(4.93)

[a] Standard error of the difference.
* Significantly different from Control, $P < 0.05$.
** Significantly different from atropine alone, $P < 0.05$.
*** Significantly different from PGE_2 alone, $P < 0.05$.

ent from one another in any of the experiments. When the same experiments were repeated in individual sheep, blood pressure and heart rate responses to the test agents given were highly repeatable. Infusion of PGE_2, 10 ng/kg/min IC, caused gradual rises in blood pressure and heart rate beginning approximately 5 min after the start of the infusion and which leveled off 15–20 min later.

Muscarinic receptor blockade. Effectiveness of muscarinic receptor blockade was determined in the following manner. For each sheep, a control dose of acetylcholine which would stop the heart for 2–5 sec was found. Among all sheep this dose ranged from 0.01 to 0.02 mg/kg iv, but once the correct dose for each sheep was found, it did not vary from day to day. Five and fifteen minutes after administration of atropine, a dose of ACH two times the control dose produced no change in heart rate. At 45 min after administration of atropine, 10 times the control dose of ACH was given. At no time did this produce a decrease in heart rate. Further evidence that 1 mg/kg atropine methyl bromide iv results in adequate muscarinic blockade was obtained in separate experiments in four of these sheep. Forty minutes after the initial bolus of atropine, additional boluses of 0.5 or 1 mg/kg atropine methyl bromide iv resulted in no additional increases in heart rate.

In eight conscious sheep, heart rate increased 26 bpm ($P < 0.05$) 5 min after atropine, and remained elevated 30 min after injection (Table I and Fig. 1B). Prostaglandin E_2 infusion, 10 ng/kg/min IC, increased heart

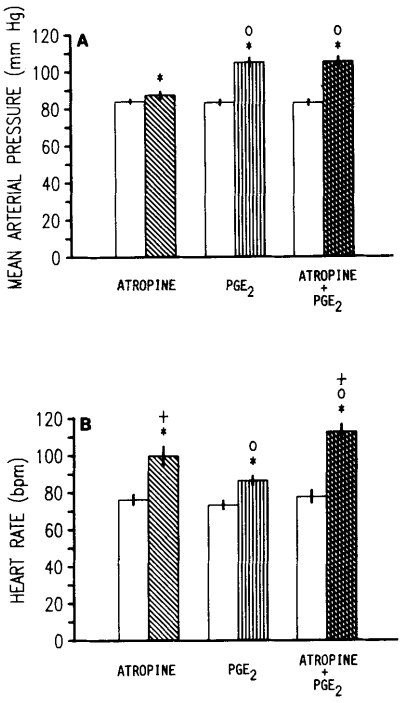

FIG. 1. Atropine + PGE_2, eight conscious sheep. Blood pressure and heart rate in three separate experiments (all experiments performed in all sheep) comparing the responses to 1 mg/kg atropine iv given alone, to 10 ng/kg/min PGE_2 IC given alone, and to atropine + PGE_2 given together. Open bars represent control values. Shaded bars represent test values 30 min after atropine alone (atropine), 25 min after the start of PGE_2 infusion alone (PGE_2), and 30 min after atropine and 25 min after the start of PGE_2 infusion when the two drugs were given together (atropine + PGE_2). (A) Blood pressure: * = $P < 0.05$ re control; O = $P < 0.05$ re atropine. (B) Heart rate: * = $P < 0.05$ re control; O = $P < 0.05$ re atropine; + = $P < 0.05$ re PGE_2.

TABLE II. HEART RATE INCREASES TO TEST BOLUSES OF ISOPROTERENOL, 5 μg BEFORE AND 50 μg AFTER EXPERIMENTAL AGENTS, DURING FOUR EXPERIMENTAL PROTOCOLS IN SEVEN CONSCIOUS SHEEP

Experimental protocol	Heart rate increase (bpm)	
	Isoproterenolol, 5 μg before propranolol (mean ± SEM)[a]	Isoproterenolol, 50 μg after propranolol (mean ± SEM)
Propranolol, alone	68 ± 2.74	9 ± 1.24
PGE_2, then propranolol	68 ± 4.54	6 ± 1.29
Propranolol, then PGE_2	72 ± 5.38	9 ± 2.46
Propranolol, then atropine, then PGE_2	65 ± 7.69	10 ± 1.77

[a] Standard error of the mean.

rate 14 bpm ($P < 0.05$, Table I and Fig. 1B). When atropine was given after PGE_2 infusion had raised heart rate, there was a further 24 bpm increase in heart rate ($P < 0.05$), resulting in a total increase of 38 bpm from control ($P < 0.05$, Table I). When PGE_2 was infused after atropine, heart rate increased a total of 35 bpm ($P < 0.05$), which was significantly different from the increases in heart rate with atropine alone or with PGE_2 alone (Table I and Fig. 1B).

Atropine alone caused small but significant increases in blood pressure 5 and 30 min after injection ($P < 0.05$, Table I and Fig. 1A). Infusion of PGE_2 increased blood pressure 22 mm Hg ($P < 0.05$, Table I and Fig. 1A). Injection of atropine during steady-state PGE_2 infusion caused no further increase in blood pressure (Table I). When the PGE_2 infusion was started 5 min after atropine had caused blood pressure to increase, there was a further significant increase in blood pressure, but the blood pressure reached at steady state was no greater than the blood pressure caused by PGE_2 infusion alone (Table I and Fig. 1A).

The effect of atropine on the blood pressure and heart rate responses to IC PGE_2 was the same regardless of whether atropine was given before or after PGE_2.

β-Adrenoceptor blockade. To test the effectiveness of β-adrenoceptor blockade, a 5 μg bolus of isoproterenol was given iv during control. At 7 and 15 min after the initial bolus of propranolol, 25 μg isoproterenol iv had no effect on heart rate. At 45 min after propranolol, the heart rate responses to 10 times the control dose of isoproterenol (50 μg) were compared to the control responses (Table II).

In seven conscious sheep, propranolol alone did not change heart rate (Table III and Fig. 2). Prostaglandin E_2 infusion in these sheep increased heart rate 15 bpm ($P < 0.05$, Table III and Fig. 2). When PGE_2 had raised heart rate, subsequent administration of propranolol decreased heart rate 9 bpm ($P < 0.05$), but heart rate still was greater than control ($P < 0.05$, Table III). When propranolol was given before the start of PGE_2 infusion, PGE_2 still increased heart rate 7 bpm ($P < 0.05$), but this increase was less than the increase caused

TABLE III. β-ADRENOCEPTOR BLOCKADE WITH PROPRANOLOL (1 mg/kg + 0.025 mg/kg/min iv) ALONE, DURING, OR BEFORE IC PGE_2 INFUSION (10 ng/kg/min) IN SEVEN CONSCIOUS SHEEP

Experimental protocol	Blood pressure (mm Hg)			Heart rate (bpm)		
	Control	Experimental	SED[a]	Control	Experimental	SED
PGE_2 (25 min)	84	105*	(1.63)	77	92*	(2.19)
Propranolol (10 min)	85	85	(0.34)	76	72	(1.65)
(35 min)	84	83	(0.58)	75	73	(2.01)
PGE_2 (25 min) + propranolol (10 min)	87	109*	(2.90)	78	83*,**	(1.86)
Propranolol (35 min) + PGE_2 (25 min)	85	106*	(1.77)	77	84*,**	(2.11)

[a] Standard error of the difference.
* Significantly different from control and propranolol alone, $P < 0.05$.
** Significantly different from PGE_2 alone, $P < 0.05$.

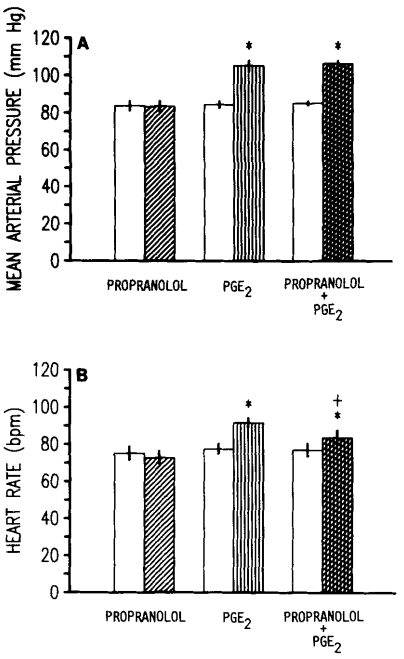

FIG. 2. Propranolol + PGE_2, seven conscious sheep. Blood pressure and heart rate in three separate experiments (all experiments performed in all sheep) comparing the responses to 1 mg/kg + 0.025 mg/kg/min propranolol iv given alone, to 10 ng/kg/min PGE_2 IC given alone, and to propranolol + PGE_2 given together. Open bars represent control values. Shaded bars represent test values 35 min after propranolol alone (propranolol), 25 min after the start of PGE_2 infusion alone (PGE_2), and 35 min after propranolol and 25 min after the start of PGE_2 infusion when the two drugs were given together (propranolol + PGE_2). (A) Blood pressure: * = $P < 0.05$ re control and propranolol. (B) Heart rate: * = $P < 0.05$ re control and propranolol; + = $P < 0.05$ re PGE_2.

by PGE_2 infusion alone ($P < 0.05$, Table III and Fig. 2). The effect of propranolol on the heart rate responses to IC PGE_2 was the same regardless of whether propranolol was given before or after PGE_2.

In these sheep PGE_2 increased blood pressure 21 mm Hg ($P < 0.05$). Propranolol had no effect on control blood pressure or on the blood pressure response to IC PGE_2.

Combined blockade. Propranolol by itself did not change heart rate, but heart rate increased 17 bpm ($P < 0.05$) when atropine was added (Fig. 3). Subsequent infusion of IC PGE_2 caused no further increase in heart rate. When all three drugs were given together, heart rate was not different from heart rate during atropine alone.

The combination of propranolol and atropine did not alter the blood pressure response to subsequent PGE_2 infusion.

Discussion. This study was performed to determine the mechanism of the tachycardia which accompanies the central pressor effect of IC PGE_2 in conscious sheep. In the first series of experiments presented here, muscarinic receptor blockade did not affect the heart rate response to IC PGE_2. Propranolol reduced, but did not abolish, the PGE_2-induced tachycardia. Although small, this remaining increase in heart rate during β-adrenoceptor blockade is significant, especially since the parasympathetic nervous system's ability to lower heart rate in response to the PGE_2-induced increase in blood pressure is unopposed.

Recently, a nonadrenergic-nonmuscarinic mechanism of increased heart rate following increases in afterload has been reported (19, 20). To determine whether this mechanism contributes to the change in heart rate during IC PGE_2 infusion, combined blockade with both propranolol and atropine was performed prior to IC PGE_2 infusion. In these experiments PGE_2 did not raise heart rate beyond the level obtained during propranolol and atropine.

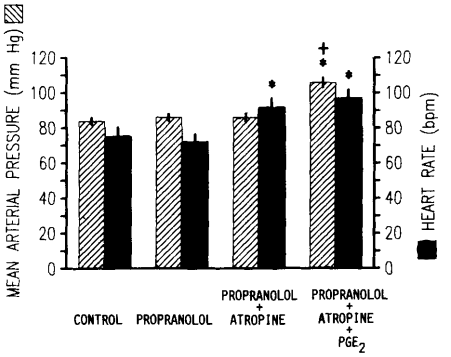

FIG. 3. Propranolol (1 mg/kg + 0.025 mg/kg/min iv) + atropine (1 mg/kg iv) + PGE_2 (10 ng/kg/min IC) in seven conscious sheep. Bars represent values of blood pressure and heart rate during consecutive administration of substances during one experiment. Note that administration of propranolol did not change heart rate or blood pressure. Addition of atropine 5 min later caused a significant increase in heart rate with no change in blood pressure. Subsequent infusion of IC PGE_2 caused no further increase in heart rate and raised blood pressure. * = $P < 0.05$ re control and propranolol. + = $P < 0.05$ re propranolol + atropine.

Since combined β-adrenergic and muscarinic blockades abolish the PGE_2-induced tachycardia, and since α-adrenergic blockade prevents the PGE_2-induced increase in blood pressure (7), we conclude that the increases in blood pressure and heart rate during IC PGE_2 infusion, 10 ng/kg/min in conscious sheep, are caused by peripheral sympathetic activation and by parasympathetic inhibition. Furthermore, it appears that during blockade of only one division of the autonomic nervous system, IC PGE_2 increases heart rate by means of the other unblocked division. This mechanism is sensitive to blockade by propranolol alone but not by atropine alone, suggesting that heart rate is preferentially increased by sympathetic activation during IC PGE_2 infusion.

The central pressor and tachycardic effects of PGE_2 also have been studied in rats (11–15). Our results in sheep are in agreement with the results of Hoffman and Schmid in rats (11), who also found both sympathetic activation and parasympathetic inhibition in response to centrally administered PGE_2. These data, together with data from previous studies in which IC PGE_2 reset the baroreflex (7) without acting directly at the carotid sinus (6, 9), support the hypothesis that IC PGE_2 acts within the central baroreflex pathway.

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1. Abdel-Halim MS, Lunden I, Cseh G, Anggard E. Prostaglandin profiles in nervous tissue and blood vessels of the brain of various animals. Prostaglandins **19**(2):249–258, 1980.
2. Hagen AA, White RP, Robertson JT. Synthesis of prostaglandins and thromboxane B_2 by cerebral arteries. Stroke **10**(3):306–309, 1979.
3. Skarnes RC, Brown SK, Hull SS, McCraken JA. Role of prostaglandin E in the biphasic fever response to endotoxin. J Exp Med **154**:1212–1224, 1981.
4. Takahashi H, Bunag RD. Centrally induced cardiovascular and sympathetic nerve responses to bradykinin in rats. J Pharmacol Exp Ther **216**:192–197, 1981.
5. Moncada S, Flower RJ, Vane JR. Prostaglandins, prostacyclin and thromboxane A_2. In: Gilman AG, Goodman LS, Gilman A, eds. The Pharmacological Basis of Therapeutics. New York, Macmillan, p673, 1980.
6. Breuhaus BA, Chimoskey JE. PGE_2 does not act at carotid sinus to raise arterial pressure in conscious sheep. Amer J Physiol **245**:H1007–H1012, 1983.
7. Hull SS Jr, Chimoskey JE. Mechanisms of central prostaglandin E_2 hypertension in conscious dogs, sheep and calves. Amer J Physiol **247**:H218–H228, 1984.
8. Yamamoto M, Share L, Shade RE. Vasopressin release during ventriculo-cisternal perfusion with prostaglandin E_2 in the dog. J Endocrinol **71**:325–331, 1976.
9. McQueen DS, Belmonte C. The effects of prostaglandins E_2, A_2 and F_{2alpha} on carotid baroreceptors and chemoreceptors. Q J Exp Physiol **59**:63–71, 1984.
10. Chimoskey JE, Breuhaus BA. Intraventricular PGE_2 raises arterial pressure less than intracarotid PGE_2 raises arterial pressure in conscious sheep. Fed Proc **44**(3):815, 1985. [Abstract]
11. Hoffman WE, Schmid PG. Cardiovascular and antidiuretic effects of central prostaglandin E_2. J Physiol **288**:159–169, 1979.
12. Kondo K, Okuno T, Saruta T, Kato E. Effects of intracerebroventricular administration of PGI_2, PGE_2, and PGF_{2alpha} and indomethacin on blood pressure in the rat. Prostaglandins **17**:769–773, 1979.
13. Hoffman WE, Leavitt ML, Albrecht RF, Miletich DJ. Central cardiovascular activity of PGE_2, PGF_{2alpha}, and PGI_2. Prostaglandins **21**:899–904, 1981.
14. Takahashi H, Bunag RD. Pressor responses to centrally-administered PGE_2 in spontaneously hypertensive rats. Hypertension **3**:426–432, 1981.
15. Okuno T, Lindheimer MD, Oparil S. Central effects of PGE_2 on blood pressure and plasma renin activity in rats—Role of the sympathoadrenal system and vasopressin. Hypertension **4**:809–816, 1982.
16. Herd JA, Barger AC. Simplified technique for chronic catheterization of blood vessels. J Appl Physiol **19**: 791–792, 1964.
17. Weiner N. Atropine, scopalamine, and related antimuscarinic drugs. In: Gilman AG, Goodman LS, Gilman A, eds. The Pharmacological Basis of Therapeutics. New York, Macmillan, p121, 1980.
18. Weiner N. Drugs that inhibit adrenergic nerves and block adrenergic receptors. In: Gilman AG, Goodman LS, Gilman A, eds. The Pharmacological Basis of Therapeutics. New York, Macmillan, p190, 1980.
19. Evans JM, Rountree R, Randall D, Knapp C, Gillespie MN. Nonadrenergic nonmuscarinic cardioacceleration: Impact of drug and hydraulically-induced increases in afterload. Physiologist **27**(4):225, 1984. (Abstract)
20. Rigel DF, Lipson D, Katona PG. Excess tachycardia: Heart rate after antimuscarinic agents in conscious dogs. Amer J Physiol **246**:H168–H173, 1984.

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