Cholinergic and β-adrenergic control of cardiovascular reflex responses to brief repeated asphyxia in term-equivalent fetal sheep

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Abstract

The role of cholinergic and β-adrenergic activity in mediating fetal cardiovascular recovery from brief repeated episodes of asphyxia consistent with established labor, remains unclear. In this study, we tested the effect of cholinergic and β-adrenergic blockade on the fetal chemoreflex and fetal heart rate (FHR) overshoot responses during brief repeated asphyxia at rates consistent with early or active labor. Chronically instrumented fetal sheep at 0.85 of gestation received either i.v. atropine sulfate (cholinergic blockade, n=8) or vehicle (n=7) followed by 3 x 1-minute umbilical cord occlusions repeated every 5 minutes (1:5; consistent with early labor), or i.v. propranolol hydrochloride (β-adrenergic blockade, n=6) or vehicle (n=6) followed by 3 x 2-minute occlusions repeated every 5 minutes (2:5; consistent with active labor). In vehicle-controls, 1:5 occlusions were associated with rapid and sustained FHR decelerations followed by rapid return of FHR to baseline values after release of the occlusion. Cholinergic blockade abolished FHR decelerations during occlusions and caused FHR overshoot after release of the occlusion ($P<0.05$ vs. control 1:5). In vehicle-controls, 2:5 occlusions caused rapid and sustained FHR decelerations followed by FHR overshoot after release of the occlusion. β-adrenergic blockade was associated with greater reduction in FHR during occlusions and attenuated FHR overshoot ($P<0.05$ vs. control 2:5). These data demonstrate that the FHR overshoot pattern after asphyxia is mediated by a combination of attenuated parasympathetic activity and increased β-adrenergic stimulation of the fetal heart.

Introduction

Fetal heart rate (FHR) monitoring is widely used to non-invasively and continuously monitor fetal wellbeing in labor. Although normal FHR recordings are highly reassuring, i.e. the negative predictive value is strong, the positive predictive value for acidosis or other complications is very low (6, 30). The most characteristic changes in FHR during labor are the recurrent rapid falls in FHR associated with uterine contractions known as intrapartum decelerations (29). In some cases, a rapid increase in FHR above baseline can be seen immediately after the deceleration (43). This pattern of FHR deceleration followed by rapid acceleration above baseline is referred to as heart rate overshoot. This overshoot pattern has been described in fetuses that were subsequently depressed at birth (15) or later developed cerebral palsy (39), suggesting that it might have diagnostic utility.

There is little information on the specific mechanisms of overshoot. There is some evidence that FHR overshoot may reflect progressive fetal acidosis and impaired cerebral metabolism (19, 34, 38). However, in fetal sheep overshoot can occur well before the onset of fetal compromise (43), strongly inferring an autonomic mechanism, such as a combination of asphyxia-induced inhibition of vagal tone and unopposed β-adrenergic stimulation of the myocardium (43). Parasympathetic inhibition of heart rate (14) is mediated through the M2 muscarinic receptors on cardiac pacemaker cells (41). Although the impact of parasympathetic blockade during brief asphyxia is unknown, it is well established that the initial FHR deceleration during brief asphyxia is a chemoreflex and mediated by parasympathetic efferents, as recently reviewed (29). Conversely, β1-adrenergic receptor activation is

61 critical to maintain FHR during hypoxia (7, 37); α-adrenergic activity has no direct
62 effect on the fetal heart (37).

63 Systematic studies in term-equivalent fetal sheep found that during intermittent brief
64 asphyxia induced by umbilical cord occlusion the precise duration of occlusion was
65 critical to whether overshoot occurred or not (43). We found that 1-minute umbilical
66 cord occlusions repeated every 5 minutes did not trigger FHR overshoot, whereas it
67 occurred at the very start of a series of 2-minute occlusions repeated every 5 minutes
68 (43). We have recently shown that overshoot is not attenuated by sympathectomy
69 (28). Nevertheless, levels of circulating catecholamines increase very rapidly during
70 brief asphyxia, and thus increase β-adrenergic activity independent of the neural
71 sympathetic system (13, 24).

72 In the present study we first tested the hypothesis that lack of overshoot after 1 min of
73 umbilical cord occlusion was due to continued vagal tone, and therefore that infusion
74 of the M2 receptor antagonist atropine to near term fetal sheep exposed to 1-minute
75 occlusions every 5 minutes (1:5) would unmask FHR overshoot. We then tested
76 whether β-adrenoreceptor blockade with propranolol would prevent or attenuate FHR
77 overshoot after 2-minute occlusions repeated every 5 minutes (2:5) to test the
78 hypothesis that the asphyxia-induced increase in circulating catecholamines
79 stimulates FHR overshoot.

80

Methods

Surgical procedures

All procedures were approved by the Animal Ethics Committee of the University of Auckland. Twenty-seven Romney/ Suffolk sheep (119-126 days gestation; term=147 days) were operated on using sterile techniques. Food, but not water was withdrawn 18 h before surgery. Ewes were given oxytetracycline (20 mg/kg, Phoenix Pharm, Auckland, New Zealand) intramuscularly 30 min before surgery for prophylaxis, to reduce the risk of post-surgical infection. General anesthesia was induced by intravenous (i.v.) injection of propofol (5 mg/kg, AstraZaneca Limited, Auckland, New Zealand), and maintained using 2-3% isoflurane (Medsource Ltd., Ashburton, New Zealand) in O_2. During surgery, ewes received an i.v. infusion of isotonic saline (250 mL/h) to maintain fluid balance and the depth of anesthesia, maternal heart rate and respiration were continuously monitored by trained anesthetic staff.

Instrumentation

A paramedian abdominal incision was made and the fetal head was exposed through a uterine incision. Polyvinyl catheters were inserted in the right and left brachial artery, brachial vein and amniotic cavity. A pair of electrodes was sewn over the fetal chest to measure the fetal electrocardiogram (ECG). An inflatable silicone occluder (In Vivo Metric, Healdsburg, CA, USA) was placed loosely around the umbilical cord near its abdominal insertion. All fetal leads were exteriorized through the maternal flank. Antibiotics (Gentamycin; 80 mg; Pfizer New Zealand, Auckland, New Zealand) were administered into the amniotic sac before closure of the uterus. A

maternal long saphenous vein was catheterized to provide access for post-operative care.

Sheep were housed in separate metabolic cages with access to water and food *ad libitum* in a temperature-controlled room (16 ± 1°C, humidity 50 ± 10%) with a 12 h light dark cycle. Five days of post-operative recovery was allowed before experiments. During this time, ewes received intravenous antibiotics daily for 4 days (gentamycin; 80 mg and benzylpenicillin sodium; 600 mg; Novartis, Auckland, New Zealand). Fetal catheters were maintained patent by continuous infusion of heparinized saline (20 IU/mL) at a rate of 0.2 mL/h.

Experimental recordings

Fetal mean arterial blood pressure (MAP) and ECG were recorded continuously for offline analysis using custom data acquisition software (LabView for Windows, National Instruments, Texas, USA). The blood pressure signal was recorded with Novatrans III Gold pressure transducers (Medex Inc., Hilliard, OH, USA), corrected for movement by subtraction of amniotic pressure, and collected at 64 Hz and low pass filtered at 30 Hz. The fetal ECG was analog filtered between 0.05 and 100 Hz and digitized at 512 Hz and used to derive FHR.

Experimental protocol

Experiments were conducted at 124-130 days gestation, when neural development approximates that of the term human infant (3, 32). Fetuses received an intravenous infusion of the M2 receptor antagonist atropine (n=8, atropine sulfate, Sigma-Aldrich, Auckland, New Zealand; 4.8 mg bolus followed by a 4.8 mg/h over 30 min) or the non-selective β-adrenoreceptor antagonist propranolol (n=6, propranolol

hydrochloride, Sigma-Aldrich, Auckland, New Zealand; 5 mg bolus followed by 5 mg/h over 30 min). Vehicle-controls received an equivalent volume of intravenous isotonic saline. Group allocations (treatment vs. vehicle control) and treatment (propranolol vs. atropine) were randomly assigned. Intravenously administered propranolol and atropine both have a half-life of approximately 2 h (18, 40).

Infusions were started 15 min before occlusions and maintained until the end of the occlusion series. Total umbilical cord occlusions were performed by rapid complete inflation of the occluder with a known volume of saline. Atropine treated fetuses underwent three 1-minute umbilical cord occlusions repeated every 5 minutes (3 x 1:5) and propranolol treated fetuses underwent three 2-minute occlusions repeated every 5 minutes (3 x 2:5). Vehicle controls received either 3 x 1:5 occlusions (n=7) or 3 x 2:5 occlusions (n=6). Fetal arterial blood gas analysis (ABL 800, Radiometer, Copenhagen, Denmark) and measurements of glucose and lactate (YSI 2300, Yellow Springs Instruments, OH, USA) were performed immediately before the first occlusion and immediately after the end of the third occlusion. At the end of the experiment, ewes and fetuses were killed by an overdose of pentobarbital sodium (9 g i.v. to the ewe; Pentobarb 300, Chemstock International, Christchurch, New Zealand).

Data analysis and statistics

To enable accurate assessment of the immediate adaptive phase of the chemoreflex during umbilical cord occlusions, 5 s averages of MAP and FHR were derived for each fetus. The rate of change in MAP and FHR during umbilical cord occlusion were derived by calculating the slope (y) for each of the variables, where y is the difference in pressure or FHR/ total duration of the occlusion (min) in the 1:5 groups or the final minute of occlusion in the 2:5 groups. Fetal heart rate overshoot height was defined as

the maximum acceleration in FHR immediately after the occlusion (within the first 30 s after release of the occluder) that was 15 beats or more above the baseline FHR before occlusion.

Statistical analyses were undertaken using SPSS (v22, SPSS, IL, USA) and Sigmaplot software (v12, SYSTAT, IL, USA). Between and within group comparisons of fetal blood gases, glucose, lactate, FHR and MAP were performed by two-way repeated measures ANOVA. Physiological data for each occlusion and recovery (interocclusion) period were analyzed individually. When statistical significance was found between groups or between group and time, post hoc comparisons were made using a Fisher's Least Significant Difference test. Mann-Whitney U-tests were used for testing non-parametric data. Statistical significance was accepted when $P < 0.05$.

Results

Before occlusions

Baseline fetal pH, blood gases, glucose and lactate concentrations did not differ between groups (Tables 1 and 2). Atropine infusion caused a transient increase in FHR (from 161±6 to 199±8 bpm; $P<0.05$) that returned to baseline values before occlusion. Propranolol infusion reduced FHR (from 170±7 to 142±3 bpm; $P<0.05$) until occlusions began. Before occlusions, there was no effect of infusion on MAP in the atropine (pre-infusion 45±1 vs. post-infusion 46±2 mmHg) or propranolol (pre infusion 43±1 vs. post infusion 44±2 mmHg) groups.

Effect of cholinergic blockade on the cardiovascular adaptation during 1:5 occlusions

In the vehicle controls, umbilical cord occlusions were associated with rapid bradycardia and hypertension (Figures 1A and B). In atropine treated fetuses, FHR was markedly higher compared to controls during all three occlusions (Figure 1A; $P<0.05$). In atropine treated fetuses, a small transient increase in FHR was seen early after the start of the first two occlusions. During the latter stage of the second and third occlusions (within 54±1 and 50±4 seconds from the start of the occlusion, respectively), a small reduction in FHR was observed in the atropine group (37±8 and 53±13 bpm from baseline, respectively). Furthermore, during occlusions the rate of reduction in FHR was slower in atropine treated fetuses compared to controls (Figure 2A; $P<0.05$). The absolute and rate of increase in MAP was higher in the atropine group compared to controls during occlusions (Figure 1B and 2B; $P<0.05$).

Effect of cholinergic blockade on the interocclusion period (1:5 occlusions)

During the interocclusion periods, FHR rapidly returned to near baseline levels and MAP remained elevated above baseline in controls. In atropine treated fetuses, FHR and MAP were markedly higher than controls (Figures 1A and B; $P<0.05$). Overshoot tachycardia was not observed in controls, however all atropine treated fetuses developed overshoot tachycardia immediately after all three occlusions (Figure 5; $P<0.05$ vs. control).

Fetal arterial blood gases, glucose and lactate concentrations after 1:5 occlusions

Umbilical cord occlusions were associated with a small fall in pH and PaO_2, and increase in $PaCO_2$ and lactate that did not differ between groups (Table 1; $P<0.05$ vs. before occlusion).

Effect of β-adrenergic receptor blockade on the cardiovascular adaptation during 2:5 occlusions

In vehicle controls, umbilical cord occlusions were associated with a rapid onset bradycardia that was sustained for the first minute of occlusion. A small increase in FHR was observed during the second minute of occlusion (Figure 3A). In propranolol treated fetuses, occlusions were associated with a greater reduction in FHR throughout the first and second occlusions (Figure 3A; $P<0.05$). In controls, MAP increased rapidly during occlusions (Figure 3B). In propranolol treated fetuses, the increase in MAP was attenuated compared to vehicle controls during the first occlusion ($P<0.05$; Figure 3B). During the second and third occlusions, MAP increased during the first 15 seconds in the propranolol group and then rapidly fell below the level of controls ($P<0.05$; Figure 3B). The rate of FHR recovery during the second minute of the first occlusion was reduced in propranolol treated fetuses

compared to controls ($P<0.05$; Figure 4A); there was no significant difference between groups during the second ($P=0.08$ vs. control) and third occlusions. During the second minute of occlusions, the slope of MAP was lower in propranolol treated fetuses compared to controls ($P<0.05$; Figure 4B).

Effect of β-adrenergic receptor blockade on the interocclusion period (2:5 occlusions)

After each 2-minute occlusion, rapid onset overshoot tachycardia was consistently observed in controls, followed by progressive resolution to near baseline levels. In contrast, propranolol infusion was associated with reduced FHR compared to controls ($P<0.05$; Figure 3A), and marked attenuation of overshoot, such that after the first occlusion FHR recovered to near baseline levels. After the second and third occlusions, although a small, transient tachycardia occurred in propranolol treated fetuses, the magnitude of FHR overshoot was substantially reduced compared to controls ($P<0.05$; Figure 5B). During the interocclusion periods, MAP remained higher in propranolol treated fetuses than controls ($P<0.05$; Figure 3B).

Fetal arterial blood gases, glucose and lactate concentrations after 2:5 occlusions

In both groups, 2-minute occlusions were associated with reduced pH and increased $PaCO_2$, lactate and glucose concentrations, relative to baseline values ($P<0.05$). A greater increase in arterial lactate concentration and reduction in pH were observed in propranolol treated fetuses compared to controls ($P<0.05$; Table 2).

Discussion

Brief repeated asphyxia lasting 1 or 2 minutes was associated with a rapid chemoreflex response in intact fetuses, as shown by rapid onset bradycardia and hypertension. Occlusion for 1 minute did not induce FHR overshoot in controls, consistent with previous observations (43, 44). Cholinergic blockade with atropine nearly completely attenuated the fetal chemoreflex-mediated bradycardia during asphyxia, exaggerated the initial hypertensive response, and unmasked dramatic FHR overshoot after release of occlusion. In contrast, 2-minute occlusions were associated with FHR overshoot after release of each occlusion (12, 43, 44), which was nearly completely attenuated by β-adrenergic blockade with propranolol. β-blockade was also associated with a greater reduction in FHR during occlusions and impaired the increase in MAP during occlusions. These data provide the first systematic evidence that overshoot tachycardia immediately after asphyxia reflects a combination of attenuation of cholinergic activation with myocardial β-adrenergic stimulation.

Cardiovascular adaptation to asphyxia during cholinergic blockade

Cholinergic blockade with atropine abolished the chemoreflex-mediated bradycardia and was associated with greater hypertension during 1-minute umbilical cord occlusions, as shown by a higher MAP and a greater increase in the rate of rise of MAP in the atropine group than controls. These data confirm a central role of the parasympathetic nervous system in controlling the efferent limb of the chemoreflex during complete umbilical cord occlusion, consistent with previous observations from studies of isocapnic hypoxia (10, 14, 31, 36) and partial umbilical cord occlusion (19).

Although atropine pretreatment prevented the initial rapid onset bradycardia, we observed a small, progressive fall in FHR during the second and third occlusions. Speculatively, this may reflect a cumulative effect of asphyxia on circulating adenosine levels. Adenosine has been shown to be an integral contributor to the bradycardic response during isocapnic hypoxia, as shown by marked attenuation of the reduction in FHR in hypoxic fetal sheep exposed to the adenosine receptor antagonist, 8-(p-sulphophenyl)-theophylline (27). Alternatively, it may reflect a cumulative effect of myocardial hypoxia on cardiac function. Previous observations have shown that approximately 3 minutes or more of continuous hypoxia is associated with a sustained FHR deceleration that cannot be reversed by atropine (2, 17). Further, vagotomized fetuses show a reduction in FHR after 2-3 minutes of umbilical cord occlusion (45). The present observation of a small but significant reduction in FHR within 50 seconds of asphyxia may imply an earlier, graded onset of myocardial hypoxia than previously suggested.

In both groups, MAP increased during 1-minute occlusions. However, a greater increase in MAP was observed in atropine treated fetuses than vehicle controls. Previous studies have shown that increased MAP during brief asphyxia and isocapnic hypoxia is mediated by increased peripheral vascular tone, initially through activation of α-adrenergic efferents, followed by increased circulating vasoactive agents, including catecholamines (13, 14, 22, 25). We did not measure peripheral perfusion in this study; however it has previously been reported that atropine does not augment peripheral vasoconstriction in near term fetal sheep during hypoxia (14, 36). Combined ventricular output in the fetus is strongly related to FHR, because stroke volume is constrained (16). Therefore, increased MAP in the atropine group during

asphyxia likely reflects maintenance of FHR and combined ventricular output compared to a proportionate reduction in vehicle controls (14).

Interestingly, the difference in MAP between atropine treated fetuses and controls was attenuated during the second and third occlusions. We and others have previously shown that circulating catecholamines, such as epinephrine and norepinephrine increase exponentially within the first 2 minutes of umbilical cord occlusion (from 118 ± 20 to 44495 ± 9557 and 1401 ± 236 to 88937 ± 17374 pmol/L, respectively) and play a significant role in maintaining MAP during fetal asphyxia (13, 22). This suggests that this considerable increase in circulating catecholamines may have allowed both groups to achieve similar level of hypertension during subsequent occlusions.

Cardiovascular adaptation to asphyxia during β-adrenergic blockade

Infusion of the β-adrenergic antagonist propranolol reduced baseline FHR compared to controls, consistent with previous reports (26). During occlusions, a rapid bradycardia was observed in both groups; however, FHR remained lower in propranolol treated fetuses than controls, presumptively reflecting the negative chronotropic effect of β-adrenergic blockade (9). In controls, partial recovery of FHR was observed during the second minute of occlusion, similar to previous reports (28, 42). This evolving FHR pattern was attenuated in the propranolol group during the first and second occlusions, with a reduced rate of change in FHR during the second minute of occlusion. These data suggest that there is a further increase in myocardial β-adrenergic activity in addition to a possible gradual loss of vagal activity during the second minute of the fetal chemoreflex response to asphyxia.

During 2-minute occlusions, MAP initially increased in both groups, however, propranolol treated fetuses did not sustain the increase in MAP. This likely reflects a reduction in combined ventricular output caused by the negative chronotropic and inotropic effects associated with inhibition of myocardial β-adrenergic activity (8).

Effects of cholinergic and β-adrenergic blockade during the interocclusion period

In vehicle controls, after 1-minute occlusions, FHR rapidly but progressively returned to baseline levels. These data demonstrate that there is no significant loss of vagal tone during a brief episode of asphyxia in the absence of systemic compromise, as previously reported (43). Atropine infusion was associated with FHR overshoot immediately after 1-minute occlusions. Strikingly, the magnitude of the overshoot unmasked by atropine was highly similar to that seen after a 2-minute occlusion. Although the tachycardia gradually resolved, FHR remained elevated during the interocclusion period compared to controls. These data are consistent with observations in human fetuses during labor (33) and fetal sheep exposed to prolonged isocapnic hypoxia (17), which showed overshoot tachycardia with maternal and fetal atropine treatment, respectively. Collectively, these data indicate that loss of vagal tone is integral to the development of FHR overshoot.

After occlusions, MAP remained increased in atropine treated fetuses compared to controls. Given that stroke volume is constrained in the fetus (16), the increase in MAP in the atropine group is likely to be a product of the greater increase in FHR compared to controls, in addition to continuing relative peripheral vasoconstriction in both groups, as previously shown (13, 22).

After 2-minute occlusions, propranolol treatment markedly attenuated FHR overshoot compared to controls. Furthermore, FHR remained reduced compared to controls in propranolol treated fetuses throughout the interocclusion period. In chemically sympathectomized near-term fetal sheep, we have previously reported that sympathetic neural activation does not mediate FHR overshoot during brief repeated asphyxia (28). Collectively, these data demonstrate that the increase in circulating catecholamines is essential to enable FHR overshoot, by increasing myocardial β-adrenergic activity.

Mean arterial pressure was increased after occlusions above baseline values in both propranolol treated fetuses and controls. However, propranolol was associated with a more prolonged increase in MAP. Given that FHR was lower than controls in propranolol treated fetuses, the prolonged increased in MAP was likely mediated by peripheral vasoconstriction. Supporting this, we observed greater systemic acidosis in the propranolol group compared to controls after occlusions, as shown by a lower arterial pH and higher lactate concentration. This is consistent with previous evidence that acute infusion of propranolol in fetal sheep was associated with mixed respiratory and metabolic acidosis (1). Together these data suggest that propranolol reduced fetal cardiac output, leading to reduced placental and tissue perfusion.

We speculate that the lower MAP during occlusions reflected impaired centralization of blood flow in the propranolol group, leading to a greater rise in circulating catecholamines and so increased the duration of peripheral vasoconstriction after occlusion. Alternatively, it is possible that increased peripheral vasoconstriction in the propranolol group may have been mediated indirectly through blockade of β-2

342 receptors on blood vessels, leading to unopposed α-adrenergic mediated
343 vasoconstriction. Further studies are required to resolve this question.

344 Collectively, these observations demonstrate an integral role of cholinergic and β-
345 adrenergic activity in mediating the chemoreflex response to brief repeated asphyxia
346 at rates and durations consistent with early and active labor. The data strongly
347 confirm that the FHR overshoot pattern that can be observed after asphyxia is
348 mediated by a combination of reduced vagal activation and increased β adrenergic
349 stimulation of the fetal heart.

350 *Perspectives and significance*

351 The ability of the mammalian fetus to rapidly adapt to an asphyxial insult is crucial to
352 survival. Labor is characterized by intermittent but brief asphyxia during contractions,
353 typically lasting no more than 1 to 2 minutes. Thus, understanding the physiological
354 controls of adaptation is of fundamental importance. There is compelling evidence
355 that the chemoreflex is central to the initial rapid adaptation. The intrapartum
356 deceleration reduces myocardial work (4, 5, 22), and peripheral vasoconstriction (13,
357 14, 22) redistributes combined ventricular output to vital organs such as the brain,
358 heart and adrenals (20, 21, 23). β-adrenergic antagonists such as propranolol are used
359 to treat hypertension in pregnancy, and have been associated with reduced birth
360 weight (35). The present study highlights the critical role of β-adrenergic activation to
361 cardiovascular adaptation to the brief asphyxial episodes that are typical of active
362 labor, and supports previous studies in the sheep that β-adrenergic antagonists
363 compromised fetal adaptation to asphyxia (11). These data suggest that β-adrenergic
364 antagonists should be used with caution, if at all, in labor.

Further, there is evidence from studies of brief repeated asphyxia that chemoreflex-mediated sympathetic efferent responses become attenuated after 1 to 2 minutes of asphyxia, but are reactivated in between the episodes of asphyxia (13, 28). The present study suggests that a similar pattern is observed in the parasympathetic arm of the chemoreflex response, such that vagal activity becomes attenuated after 1 to 2 minutes of asphyxia but recovers rapidly with reperfusion. Although the evidence is indirect, the point at which vagal inhibition occurs during asphyxia may represent the point in time when myocardial hypoxia contributes to a deceleration. This would reflect the transition from a period of asphyxia that is completely compensated for by the fetus, to one that is not. A marker of this transition may be clinically useful. The present study strongly suggests that this transition is, albeit imperfectly, reflected by the development of overshoot tachycardia immediately after a deceleration. Furthermore, the onset of suppression of vagal activity in the present study is a direct function of the duration of an individual occlusion. Thus, we propose that it may have utility to help understand how fetal autonomic adaptation evolves over time.

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Disclosures

The authors declare no conflict of interest.

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532 Table 1 Fetal arterial blood gases and glucose and lactate concentration in control and atropine treated fetuses exposed to 1-minute occlusions
533 every 5 minutes (1:5).

	pH		PaO_2, mmHg		$PaCO_2$, mmHg		Lactate, mmol/L		Glucose, mmol/L	
	Control	Atropine	Control	Atropine	Control	Atropine	Control	Atropine	Control	Atropine
Before 1:5	7.41±0.01	7.41±0.01	21.3±0.6	21.6±1.6	45.7±1.0	42.2±1.6	0.9±0.0	0.9±0.0	0.9±0.1	0.8±0.1
After 1:5	7.36±0.01#	7.34±0.02#	17.5±1.1#	16.5±0.8#	48.2±1.7#	47.4±2.2#	1.9±0.4#	1.7±0.0#	1.0±0.2	1.0±0.1

534 Data are mean ± SEM. #P<0.05 vs. before occlusion within groups.

535

536

537 Table 2 Fetal arterial blood gases and glucose and lactate concentration in control and propranolol treated fetuses exposed to 2-minute occlusions

538 every 5 minutes (2:5).

	pH		PaO_2, mmHg		$PaCO_2$, mmHg		Lactate, mmol/L		Glucose, mmol/L	
	Control	Propranolol	Control	Propranolol	Control	Propranolol	Control	Propranolol	Control	Propranolol
Before 2:5	7.41±0.01	7.40±0.01	22.0±1.0	20.3±1.9	46.3±2.0	41.7±1.6	0.7±0.1	0.8±0.1	0.7±0.1	0.7±0.0
After 2:5	7.34±0.01#	7.28±0.01#*	19.7±0.6	19.7±1.2	52.2±2.1#	48.1±1.7#	1.6±0.3#	2.6±0.4#*	1.0±0.1#	1.1±01#

539 Data are mean ± SEM. *P<0.05 vs. control; #P<0.05 vs. before occlusion within groups.

Figure legends

Figure 1. Fetal heart rate (FHR; A) and mean arterial pressure (MAP; B) in control (white circles) and atropine treated fetuses (black circles) exposed to 3 x 1 minute occlusions every 5 minutes. The shaded region denotes the period of asphyxia. Data are means ± SE. *P<0.05 vs. control.

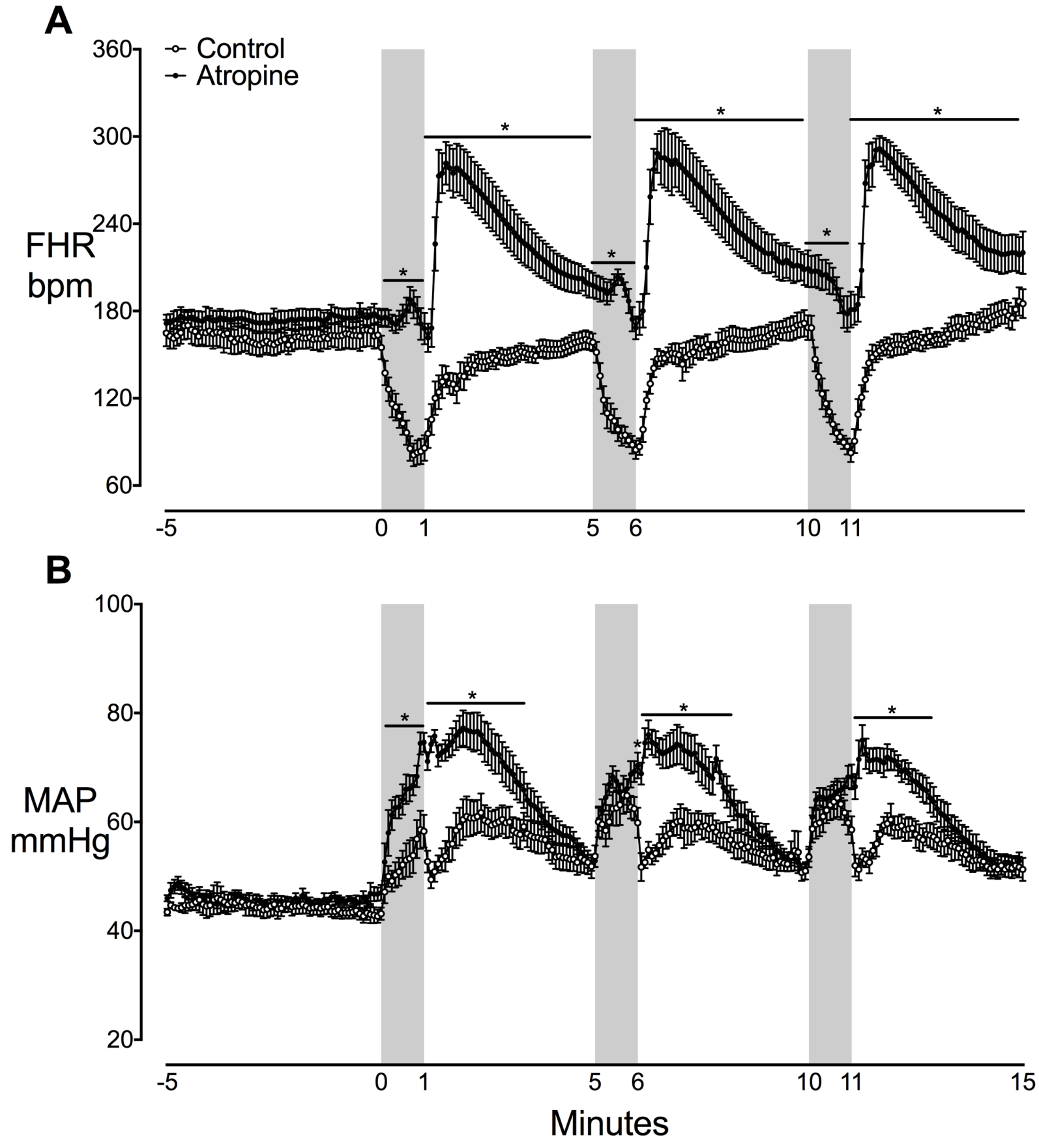
Figure 2. Rate of change (slope) of fetal heart rate (FHR; A) and mean arterial pressure (MAP; B) during the first, second and third umbilical cord occlusion in control (white bars) and atropine treated fetuses (black bars) exposed to 3 x 1 minute occlusions every 5 minutes. Data are means ± SE. *P<0.05 vs. control.

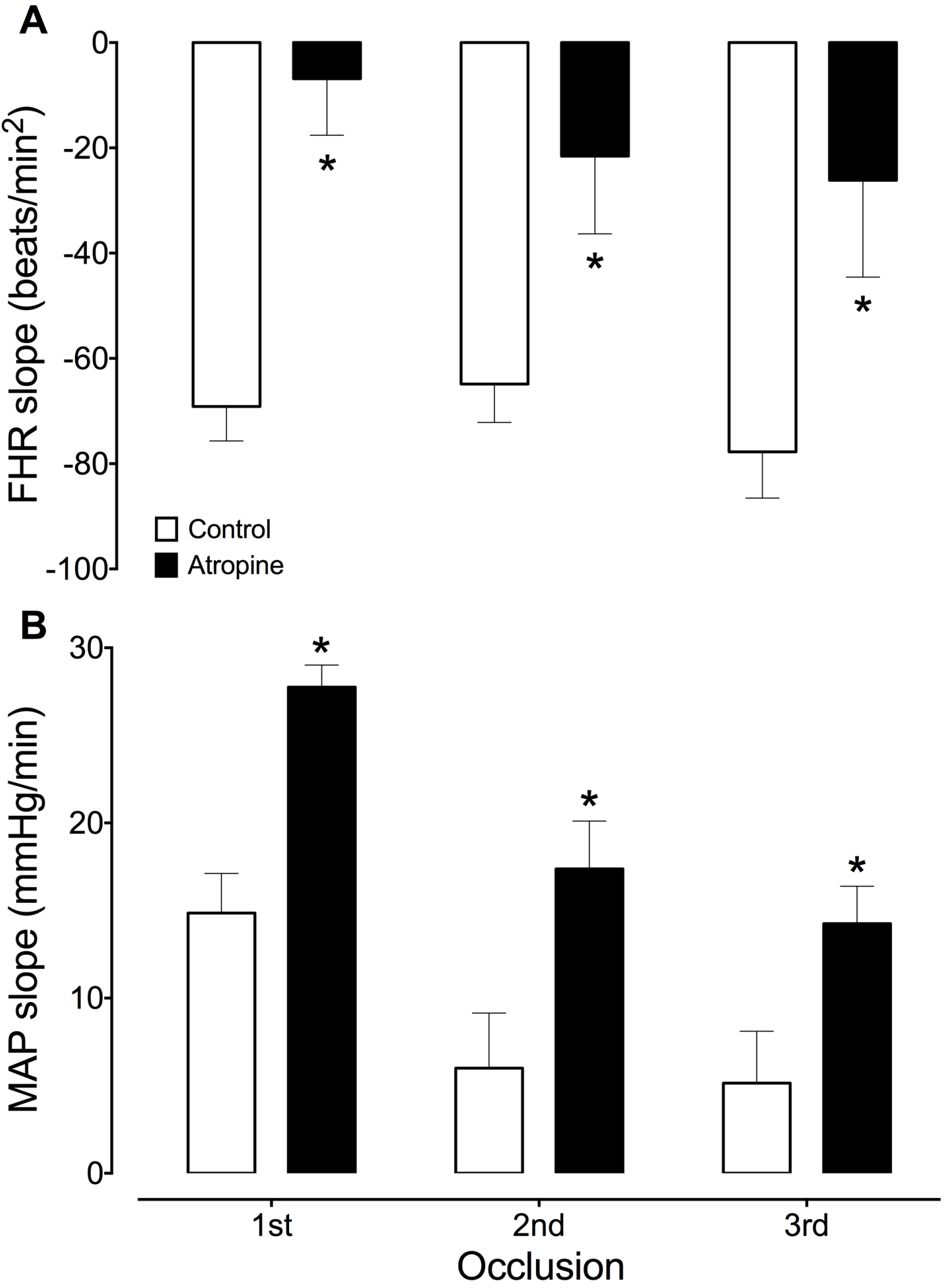
Figure 3. Fetal heart rate (FHR; A) and mean arterial pressure (MAP; B) in control (white circles) and propranolol treated fetuses (black circles) exposed to 3 x 2 minute occlusions every 5 minutes. The shaded region denotes the period of asphyxia. Data are means ± SE. *P<0.05 vs. control.

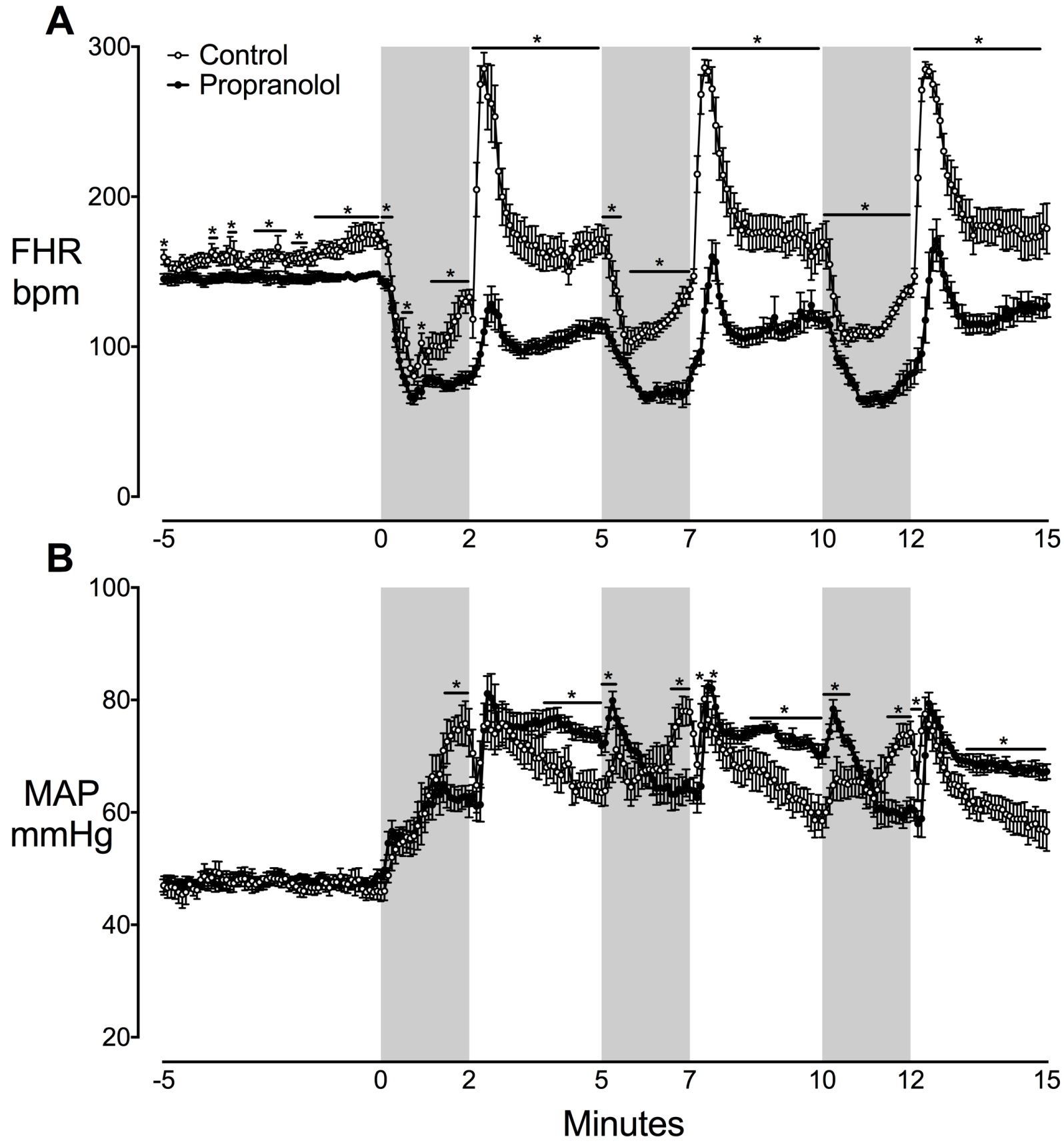
Figure 4. Rate of change (slope) of fetal heart rate (FHR; A) and mean arterial pressure (MAP; B) during the final minute of the first, second and third umbilical cord occlusion in control (white bars) and atropine treated fetuses (black bars) exposed to 3 x 2 minute occlusions every 5 minutes. Data are means ± SE. *P<0.05 vs. control, #P<0.08 vs. control.

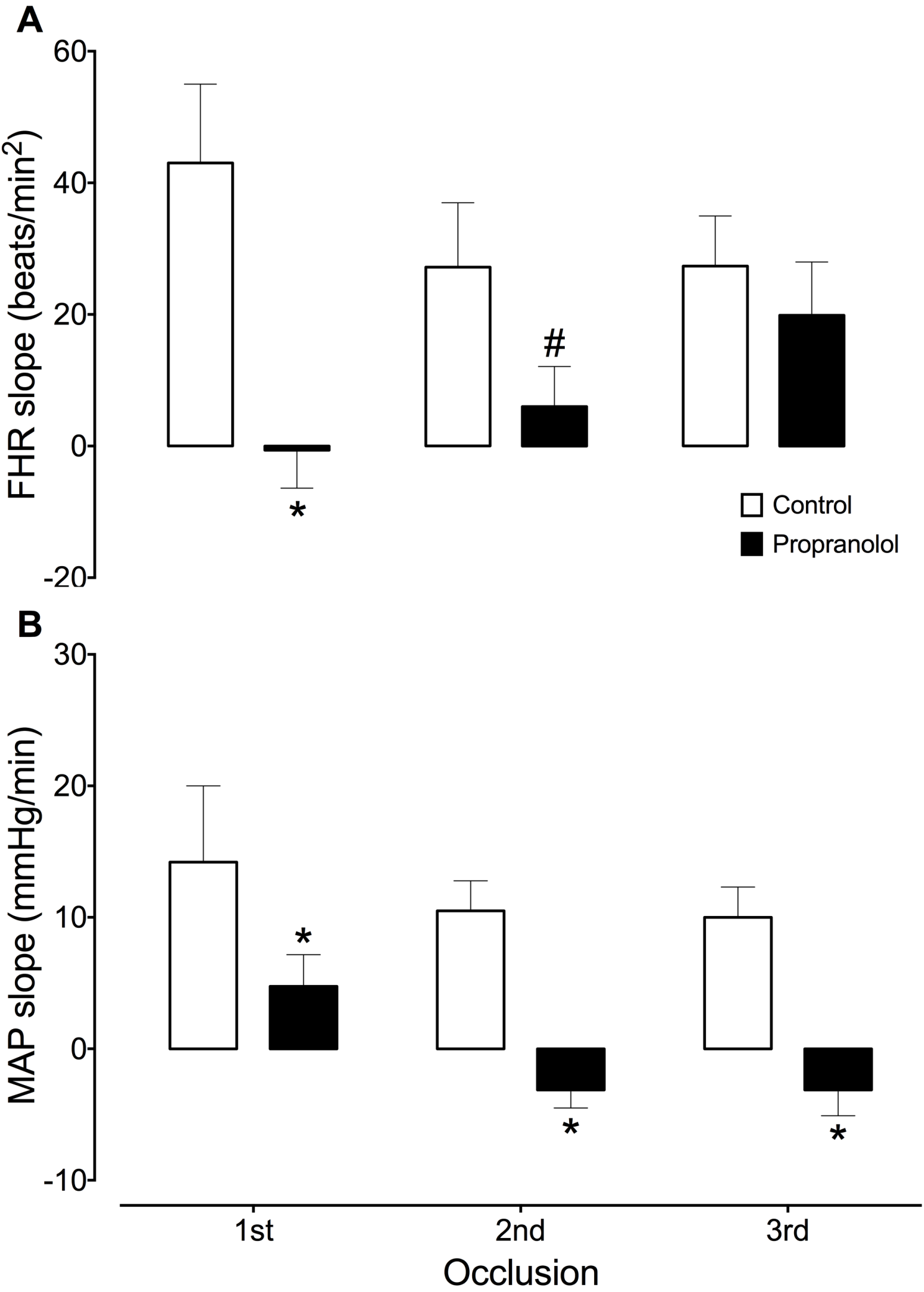
Figure 5. A: Fetal heart rate overshoot height in control (white circles) and atropine treated fetuses (black circles) during the first, second and third interocclusion period immediately after 1-minute occlusions repeated every 5 minutes. B: overshoot height in control (white circles) and propranolol treated fetuses (black circles) during the

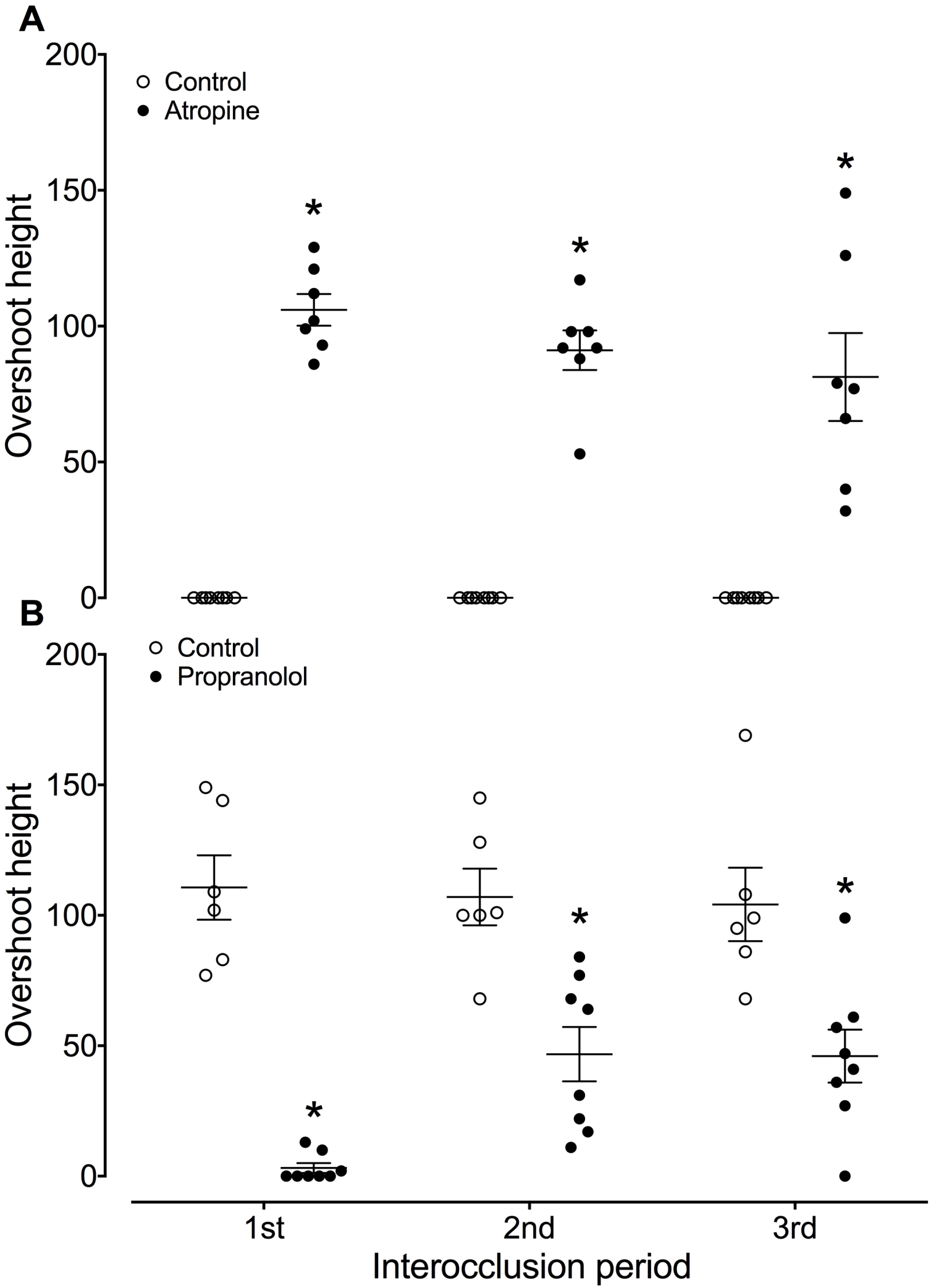
562 first, second and third interocclusion period immediately after 2-minute occlusions
563 repeated every 5 minutes. Data are means ± SE. *P<0.05 vs. control.


A
Control
Atropine
360
300
240
180
120
60
FHR
bpm
*
-5
0
1
5
6
10
11
B
100
80
60
40
20
MAP
mmHg
15
Minutes


A
0
-20
-40
-60
-80
-100
FHR slope (beats/min2)
Control
Atropine
*
*
*
B
30
20
10
0
MAP slope (mmHg/min)
*
*
*
1st
2nd
3rd
Occlusion


A
Control
Propranolol
FHR
bpm
300
200
100
0
B
MAP
mmHg
100
80
60
40
20
-5
0
2
5
7
10
12
15
Minutes


A
60
40
20
0
-20
FHR slope (beats/min2)
*
#
Control
Propranolol
B
30
20
10
0
-10
MAP slope (mmHg/min)
*
*
*
1st
2nd
3rd
Occlusion


A
200
150
100
50
0
Overshoot height
Control
Atropine
*
*
*
B
200
150
100
50
0
Overshoot height
Control
Propranolol
*
*
*
1st
2nd
3rd
Interocclusion period