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Running head: BP and vascular function following space flight

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ABSTRACT

Blood pressure and mesenteric resistance artery function were assessed in 9-week-old spontaneously hypertensive rats following an 18 day shuttle flight on STS-80. Blood pressure was measured twice, first in conscious animals using a tail-cuff method and then while the animals were anesthetized with 2% halothane in O_2. Isolated mesenteric resistance artery responses to cumulative additions of norepinephrine, acetylcholine, sodium nitroprusside, and calcium were measured within 17 hours of landing using wire myography. Blood pressure was slightly reduced in conscious animals following flight ($p=0.056$) but was significantly elevated ($p<.001$) above vivarium control group values in anesthetized animals. Maximal contraction of mesenteric arteries to norepinephrine was attenuated in the flight animals ($p<.001$) as was relaxation to acetylcholine ($p<.001$) and calcium ($p<.05$). There was no difference between flight and control animals in the vessel response to sodium nitroprusside ($p>0.05$). The results suggest that there may have been an increase in synthesis and release of nitric oxide in the flight animals.

Key words: SHR, microgravity, nitric oxide, endothelium, orthostatic intolerance

INTRODUCTION

It has become increasingly apparent that the problem of orthostatic intolerance is associated with an inability to increase peripheral resistance in the face of an orthostatic challenge (2,6). That inability may reside in the vessel itself. Studies have shown that vascular contraction is compromised and regional blood flow is altered in animals subjected to simulated weightlessness (5,11,25). If so, the animal may be less able to make the hemodynamic adjustments necessary to offset a sudden change in cardiac output.

In this report, we present the mesenteric resistance vessel results from NIH.R4, a life-sciences mission flown on STS-80. The data indicate that vessel function is altered following exposure to microgravity and suggest the hypothesis that production of nitric oxide may be responsible.

METHODS

Animals. Male spontaneously hypertensive rats (SHR) were obtained from Taconic Farms (Germantown, NY) at 21 days of age. On arrival at Kennedy Space Center, the animals were placed on either a high (2.0%) or low (0.2%) calcium diet (Teklad, Madison, WI) for the duration of the experiment. Calcium content was the only difference between the diet used and the typical space bar diet consumed by rodents on shuttle flights.

The animals were assigned to flight and vivarium control groups based on receipt date from the vendor. Shipments were offset by 3 days to allow testing of all animals at the same age. Nine rats were assigned to each treatment condition and 7 were selected for further study

based on indirect systolic blood pressure measurements at 5 and 7 weeks of age. The 7 animals with the highest blood pressure on low calcium diet and the 7 animals with the lowest blood pressure on the high calcium diet were selected.

Procedure: Two animal enclosure modules (AEM) with 7 animals each were flown on STS-80 when the animals were 7 weeks of age. One AEM contained high calcium diet and the other low calcium diet. The flight lasted 18 days. Three hours after landing the animals were available for experimentation.

A tail-cuff method (NARCO Biosystems) was used to measure indirect systolic blood pressure in all animals within one and one-half hours of receipt. Immediately after tail-cuff blood pressure was measured on the first animal, it was transferred to another room and anesthetized with halothane (2% in O_2) and a catheter was inserted into the carotid artery for measurement of direct arterial blood pressure and collection of blood. Mean arterial pressure (MAP) was measured for 5 minutes using a Statham P23-id pressure transducer in line with a Grass model 7P1 DC preamplifier (Grass Instrument Co). Data were recorded on a chart recorder before the animal was exsanguinated and the mesenteric vascular bed was collected for harvesting of resistance vessels. Thereafter one rat was sacrificed every half hour for the first 8 rats, followed by a break for 2.5 hours. The final 6 rats were also done every half hour. From beginning to end, it took a total of 14 hours to finish vessel testing.

Vessel protocol: The vascular tests were performed using branch II or III mesenteric resistance arteries (~250 μm). Upon isolation, the vessels were placed in ice-cold gassed PSS.

For testing, the vessels were mounted on a dual-channel wire myograph and bathed in

physiological salt solution (PSS) of the composition (mmol/l): NaCl 130, KCl 4.7, $NaHCO_3$ 14.9, NaH_2PO_4 1.1, Na_2EDTA 0.1, $MgSO_4 \cdot 7H_20$ 1.17, $CaCl_2$ 1.6 and glucose 5.5, with a pH of 7.4 when gassed with 95% O_2 and 5% CO_2. Normalized media thickness and lumen diameter were determined as described by Mulvany and Halpern (15). Briefly, the vessel segment was set to its initial length and, while viewed with a filar micrometer eyepiece in the field of a 40x objective, measurements of media thickness and axial length were made. These values were then used to calculate the volume of the medial layer. The vessel was then stretched to 90% of the diameter that it would have with an intraluminal pressure of 100 mmHg and the media thickness at this normalized length was calculated from the new value of the internal circumference, and the axial length and media volume (which are assumed to remain constant).

After making the morphometric measurements, the contractile response of each vessel to a challenge with 100 mmol/l KCl (NaCl substituted) was determined three times, followed by two challenges with 100 mmol/l KCl + 10 μmol/l norepinephrine. The contractile response of each vessel to the cumulative addition of norepinephrine was then determined. The apparent sensitivity of each vessel to norepinephrine was assessed using the concentration of the agonist that elicited 50% of the maximal response (EC_{50}). The EC_{50} values were determined using non-linear curve fitting. The force generated by each vessel was normalized to the cross-sectional area of the vessel and reported as active stress (mN/mm^2).

Relaxation responses to acetylcholine and sodium nitroprusside were determined by cumulative addition to vessels that were precontracted to 80% of maximal contraction with norepinephrine. Relaxation to increased extracellular calcium was tested in a low bicarbonate

PSS: NaCl 150, Kcl 5.4, NaH2PO4 1.18, $MgSO_4 \cdot 7H_20$ 1.17, $CaCl_2$ 1.6, $NaHCO_3$ 6.0, Na_2EDTA 0.03 glucose 5.5. All relaxation studies were done with $3x10^{-6}$ M indomethacin present. Relaxation was expressed as percentage of the original response to norepinephrine.

DATA ANALYSIS: Analysis of variance, with repeated measures where appropriate, was used to analyze the data for diet, treatment and dose effects. Trend analysis was used to determine whether there was any change over time in blood pressure or vessel responses as a consequence of adaptation to normal gravity. A probability of 0.05 was used to establish statistical significance.

RESULTS

Indirect systolic blood pressure before and after flight is shown in Figure 1. As expected, animals on low calcium diets had higher blood pressure than animals on high calcium diets prior to launch. The diet-induced difference in blood pressure was still evident following space flight. Systolic blood pressure was somewhat lower ($p=.057$) in the flight animals than in the control animals following flight. In contrast, MAP measured while the animals were anesthetized (Figure 2) was significantly higher in the flight animals than in the control animals ($p<.001$). There were no apparent order effects in the blood pressure data that would be indicative of a change in blood pressure over time do to readaptation to earth's gravity.

The contractile responses to NE are shown in Figure 3. Maximal contraction was significantly reduced in the flight animals relative to the control animals ($p<.001$) as was the EC50 ($p<.001$). There was no effect of diet on the vessel responses.

The relaxation response to acetylcholine is presented in Figure 4. The relaxation response was significantly attenuated in the flight animals relative to the control animals ($p < .001$). At the higher doses there was a tendency toward vasoconstriction to Ach. In contrast to acetylcholine, the response to sodium nitroprusside did not differ between flight and control animals as shown in Figure 5.

Responses to increased extracellular calcium were more variable than the those to the other relaxation conditions (Figure 6). Analysis of variance indicated that there was a significant interaction between treatment and dose ($p < .05$) due to less relaxation in the flight group at the higher calcium doses.

DISCUSSION

The major findings of the present study were that 1) blood pressure outcomes between flight and control animals differed depending on anesthetic conditions, 2) vascular contraction to NE was attenuated in the flight group, 3) relaxation to Ach was diminished in the flight group, and 4) relaxation to sodium nitroprusside was not different between groups. This constellation of outcomes is suggestive of an alteration in endothelial function that may involve increased synthesis and release of nitric oxide.

Blood pressure was lower in the flight animals than in the vivarium control animals when the animals were conscious. However, when blood pressure was measured when the animals were anesthetized with halothane, blood pressure was much higher in the flight animals than in the control animals. One interpretation of this data is that blood pressure was lower in the flight animals when conscious and higher during anesthesia because there was less

sympathetic nervous system outflow in this group. Low levels of sympathetic outflow may have led to lower blood pressure levels when conscious and resulted in less of a fall in sympathetic nervous system activity with anesthesia. However, while this interpretation is consistent with the sympatholytic effects of halothane (24), sympathetic nervous system activity is thought to be increased on return to earth's gravity (18).

Alternatively, the shift in blood pressure may have been due to the effects of halothane on endothelial-dependant vasorelaxation. Halothane inhibits the vasorelaxing effects of nitric oxide on vascular smooth muscle leading to vasoconstriction and elevated blood pressure (8). The effect of the anesthetic is very similar to giving a nitric oxide synthase inhibitor such as L-NAME (13). However, the anesthetic does not interfere with nitric oxide synthase. Instead, it appears to disrupt the nitric oxide-guanylyl cyclase signaling pathway resulting in impaired vasorelaxation and elevated blood pressure (17). This interpretation of the data actually fits well with what was observed in the vasculature.

Vascular responsiveness to NE and Ach was diminished in the flight rats but there was no change in the response to a direct vasodilator. The pattern of changes in mesenteric vascular function following space flight are the same as that which has been described for the aorta in an animal model of portal hypertension i.e., decreased maximal contraction to norepinephrine, impaired relaxation to acetylcholine and no change in response to a direct vasodilator (9). Portal hypertension is thought of as a hyperkinetic circulation state with reduced peripheral vascular resistance and elevated blood flow. The decrease in vascular resistance appears to be due to increased synthesis and release of nitric oxide.

Further support for altered endothelial function in the flight animals comes from the

data on calcium-induced relaxation. At least part of the relaxation that occurs to increasing doses of calcium is thought to be due to endothelium derived nitric oxide release (23). While there may be additional factors involved in the relaxation response to extracellular calcium, such as altered ion channel activity (1,19), the most parsimonious explanation for the vessel results observed in this study is a change in endothelial activity.

Not all of the evidence in the literature is consistent with a role for nitric oxide in the depressed vascular response to norepinephrine following exposure to microgravity. Delp et al (5), using an unweighted hindlimb model, found that removal of the vascular endothelium did not normalize the depressed response of aortic rings to vasopressors. From that observation, they concluded that it was unlikely that the depressed vascular responses were due to nitric oxide activity. While that is a reasonable conclusion, it appears that things may be more complicated than previously realized. For example, neither Karatapanis et al (9) nor Michielsen et al (14) observed normalized responses following removal of the endothelium from isolated vessels from animals with portal hypertension. However, Michielsen reported that inhibition of nitric oxide synthase with N^g-nitro-L-arginine elimninated the difference between animals with portal hypertension and control animals while Karatapanis, using nitro-L-arginine methyl ester did not.

Thus, while it is well accepted that the diminished responsiveness to norepinephrine in portal hypertension is due to overproduction of nitric oxide, there is considerable debate about the stimulus for nitric oxide production, whether the enzyme responsible is constitutive or inducible, and whether the vasculature is more or less responsive to acetylcholine (16,22). The majority contend that the primary stimulus for increased synthesis and release of nitric

oxide is increased blood flow (3) producing shear stress that stimulates synthesis and release of nitric oxide from the endothelium resulting in flow-dependant vasodilation. Given agreement on blood flow as the initiating stimulus, there is clearly much to be learned about endothelial function, nitric oxide release and vascular contractility.

An increase in blood flow and nitric oxide production may occur in microgravity. Cardiac output is increased and total peripheral resistance is decreased in the resting state in microgravity relative to erect preflight values (21). Although there is very little data available on regional blood flows during and following spaceflight, there are indications that blood flow is increased in some areas. For example, blood flow in the carotid artery is increased based on data from rhesus monkeys (20). In the renal vascular bed, glomerular filtration is increased significantly although renal plasma flow is not significantly elevated despite being higher than preflight levels (10). Following space flight, Gabrielsen et al (7) found that forearm subcutaneous vascular resistance was enhanced during lower body negative pressure. These results are very similar to those reported by Convertino et al (4) following bed rest. Together, these results suggest that different vascular beds may be exposed to very different stimuli during space flight. The centralization of fluid volume may increase blood flow to some structures while reducing it to others.

Vascular data are of paramount importance to the issue of orthostatic intolerance. Decrements in vascular responsiveness may compromise the ability of the animal to redistribute blood flow and increase total peripheral resistance when faced with a hypotensive challenge and may help explain the basis for orthostatic intolerance. Increased vascular resistance in the visceral beds is a primary means of maintaining total peripheral resistance

when faced with a sudden reduction in cardiac output. The results of NIH.R4 indicate that there is likely to be a problem increasing vascular resistance in the mesenteric vascular bed and suggests that the animal may be less able to divert blood flow from the viscera. This hypothesis is based on the observations of Woodman et al (25) and McDonald et al (11) that rats exposed to simulated weightlessness have limited ability to divert blood flow from the internal organs during exercise. Combined with the data from Buckey et al (2) and Fritsch-Yelle (6) showing that an inability to increase total peripheral resistance was the primary difference between those who develop orthostatic intolerance and those who do not, these data argue that deficits in vessel responsiveness in visceral vascular beds may contribute to orthostatic intolerance.

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References

1. Bohr, D.F. What makes the pressure go up? A hypothesis. *Hypertension* 3(supp II):II-160, 1981.

2. Buckey Jr., J.C., L.D. Lane, B.D. Levine, D.E. Watenpaugh, S.J. Wright, W.E. Moore, F.A. Gaffney, and C.G. Blomqvist. Orthostatic intolerance after spaceflight. *J. Appl. Physiol.* 1996; 81(1): 7-18.

3. Cahill, P.A. and J.V. Sitzmann. Nitric oxide and portal hypertension (editorial). *J. Hepatol.* 23(3): 355-356, 1995.

4. Convertino, V.A., D.F. Doerr, D.A. Ludwig, and J. Vernikos. Effect of simulated mirogravity on cardiopulmonary baroreflex control of forearm vascular resistance. *Am. J. Physiol.* 266:R1962-R1969, 1994.

5. Delp, M.D., T. Holder-Binkley, M.H. Laughlin, and E.M. Hasser. Vasoconstrictor properties of rat aorta are diminished by hindlimb unweighting. *J. Appl. Physiol.* 75(6):2620-2628, 1993.

6. Fritsch-Yelle, J.M., P.A. Whitson, R.L. Bondar, and T.E. Brown. Subnormal norepinephrine release relates to presyncope in astronauts after spaceflight. *J. Appl. Physiol.* 81(5): 2134-2141, 1996.

7. Gabrielsen, A., P. Norsk, R. Videbaek, and O. Henriksen. Effect of microgravity on forearm subcutaneous vascular resistance in humans. *J. Appl. Physiol.* 79(2): 434-438, 1995.

8. Jing, M., J.L. Hart, S. Bina, and S.M. Muldoon. Inhibition of nitric oxide-dependent

vasodilation by halogenated anesthetics. *Advances in Pharmacology* 31: 459-69, 1994.

9. Karatapanis, S., P.A. McCormick, S. Kakad, J.K.T. Chin, M. Islam, J. Jeremy, D. Harry, N McIntyre, A. K. Burroughs, and M. Jacobs. Alteration in vascular reactivity in isolated aortic rings from portal vein-constricted rats. *Hepatology* 20:1516-1521, 1994.

10. Leach, C.S., C.P. Alfrey, W.N. Suki, J.I. Leonard, P.C. Rambaut, L.D. Inners, S.M. Smith, H.W. Lane, and J.M. Krauhs. Regulation of body fluid compartments during short-term spaceflight. *J. Appl. Physiol.* 81(1):105-116, 1996.

11. McDonald K.S., M.D. Delp, and R.H. Fitts. Effect of hindlimb unweighting on tissue blood flow in the rat. *J. Appl. Physiol.* 72:2210-2218, 1992.

12. McIntyre, A.K. Burroughs, and M. Jacobs. Alteration in vascular reactivity in isolated aortic rings from portal vein-constricted rats. *Hepatology* 20(6): 1516-1521, 1994.

13. Meyer, J., C.W. Lentz, D.N. Herndon, S. Nelson, L.D. Traber, and D.L. Traber. Effects of halothane anesthesia on vasoconstrictor response to NG-nitro-L-arginine methyl ester, an inhibitor of nitric oxide synthesis, in sheep. *Anesthesia and Analgesia* 77(6): 1215-1221, 1993.

14. Michielsen, P.P., G.E. Boeckxstaens, S.U. Sys, A.G. Herman, and P. A. Pelckmans. The role of increased nitric oxide in the vascular hyporeactivity to noradrenaline in long-term portal vein ligated rats. *J. Hepatol.* 23: 341-347, 1995.

15. Mulvany, M.J. and W. Halpern. Contractile properties of small arterial resistance vessels in spontaneously hypertensive and normotensive rats. *Circ. Res.* 41: 19-26, 1977.

16. Niederberger, M., P. Gines, P.Y. Martin, P. Tsai, K. Morris, I. McMurtry, and R. W. Schrier. Comparison of vascular nitric oxide production and systemic hemodynamics in cirrhosis versus prehepatic protal hypertension in rats. *Hepatology* 24(4): 947-951, 1996.

17. Park, K. W., H. B. Dai, E. Lowenstein, A. Darvish, and F.W. Sellke. Isoflurance and halothane attenuate endothelium-dependent vasodilation in rat coronary microvessels. *Anesthesia and Analgesia* 84(2): 278-284, 1997.

18. Robertson, D., V. A. Convertino, and J. Vernikos. The sympathetic nervous system and the physiologic consequences of spaceflight: a hypothesis. *Am J Med Sci* 1994; 308(2): 126-132.

19. Rinaldi, G., D. F. Bohr. Potassium-induced relaxation of the arteries in hypertension: modulation by extracellular calcium. *Am J Physiol* 256:H707-H712, 1989.

20. Sandler, H., V.P. Krotov, J. Hines, V.S. Magadev, B.A. Benjamin, A.M. Badekeva, B.M. Halpryn, H.L. Stone, and V.S. Krilov. Cardiovascular results from a rhesus monkey flown aboard the Cosmos 1514 spaceflight. *Aviat. Space Environ. Med.* 58: 529-536, 1987.

21. Shykoff, B.E., L.E. Farhi, A.J. Olszowka, D.R. Pendergast, M.A. Rokitka, C.G. Eisenhardt, and R.A. Morin. Cardiovascular response to submaximal exercise in sustained microgravity. *J. Appl. Physiol.* 81(1):26-32, 1996.

22. Sogni, P., R. Moreau, A. Gadano, and D. Lebrec. The role of nitric oxide in the hyperdynamics circulatory syndrome associated with portal hypertension. *J Hepatol* 23(2): 218-24, 1995.

23. Wu, C.C. and D.F. Bohr. Mechanisms of calcium relaxation of vascular smooth muscle. *Am. J. Physiol.* 261: H1411-1416, 1991.

24. Yamahura, T., T. Kimura, and K. Furukawa. Effects of halothane, thiamylal, and ketamine on central sympathetic and vagal tone. *Anesthesia Analgesia* 62(2):129-134, 1983.

25. Woodman, C.R., L.A. Sebastian, and C.M. Tipton. Influence of simulated microgravity on cardiac output and blood flow distribution during exercise. *J. Appl. Physiol.* 79(5): 1762-1768, 1995.

Figure Legends

Figure 1. Systolic blood pressure before and after flight in each of the treatment groups. Values are mean ± SEM. The * symbol refers to a significant difference between diet groups at $P < .05$.

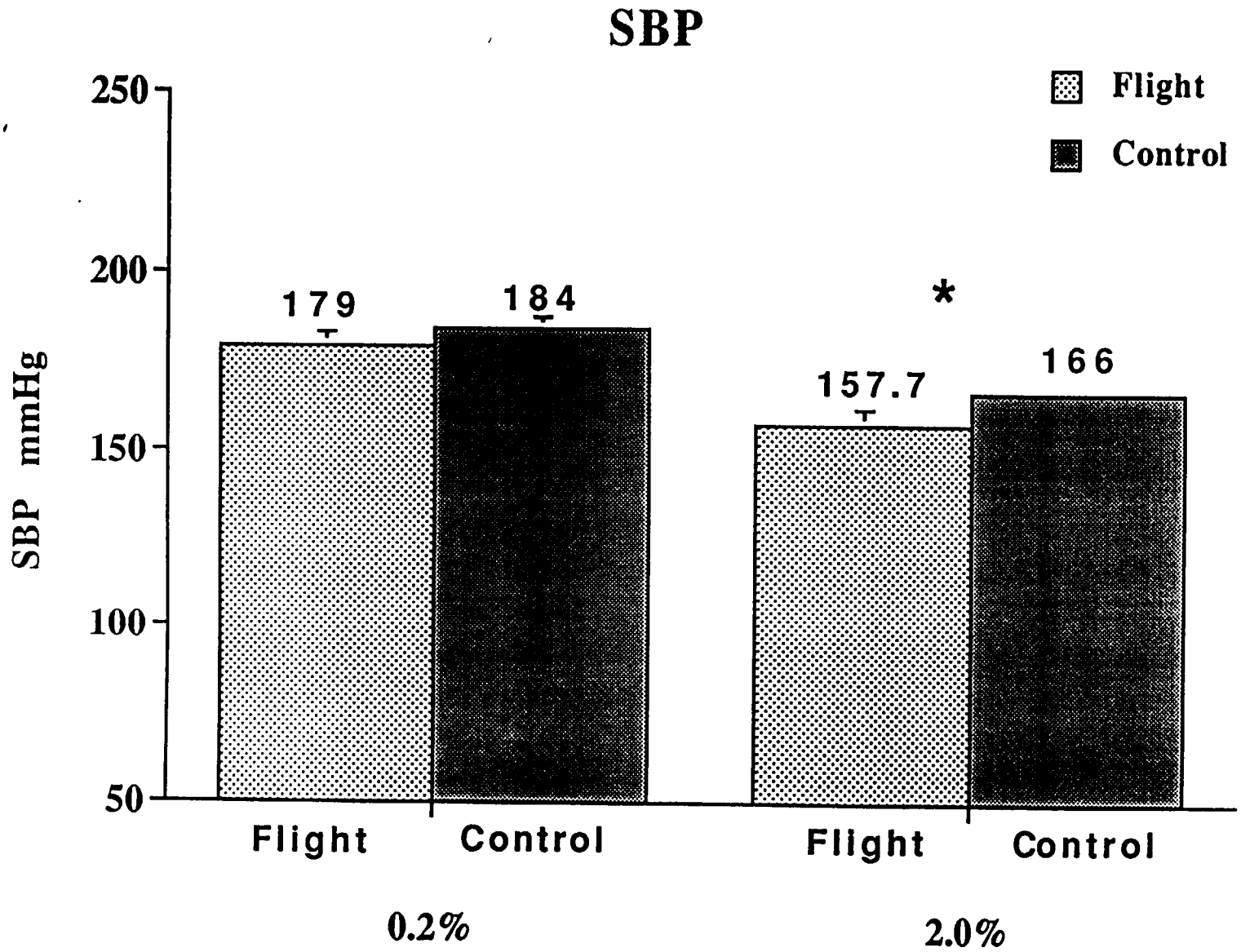
Figure 2. Mean arterial pressure before and after flight in each of the treatment groups. Values are mean ± SEM. The * symbol refers to a significant difference between diet groups at $P < .05$; the † symbol refers to a significant difference between flight and control animals at $p < .001$.

Figure 3. Contractile responses of mesenteric resistance arteries to norepinephrine. Data are for SHR flight and vivarium controls groups. Values represent means ± SEM. There were significant differences between flight and control animals ($p < .001$).

Figure 4. Relaxation responses of mesenteric resistance arteries to acetylcholine. Data are for SHR flight and vivarium control groups. Values represent means ± SEM. There were significant differences between flight and control animals ($p < .001$).

Figure 5. Relaxation responses of mesenteric resistance arteries to sodium nitroprusside. Data are for SHR flight and vivarium control groups. Values represent means ± SEM. There were no significant differences between flight and control animals ($p > .05$).

Figure 6. Relaxation responses of mesenteric resistance arteries to increasing concentrations of extracellular calcium. Data are for SHR flight and vivarium control groups. Values represent means ± SEM. There was significantly less relaxation in the flight group at higher calcium concentrations ($p < .05$).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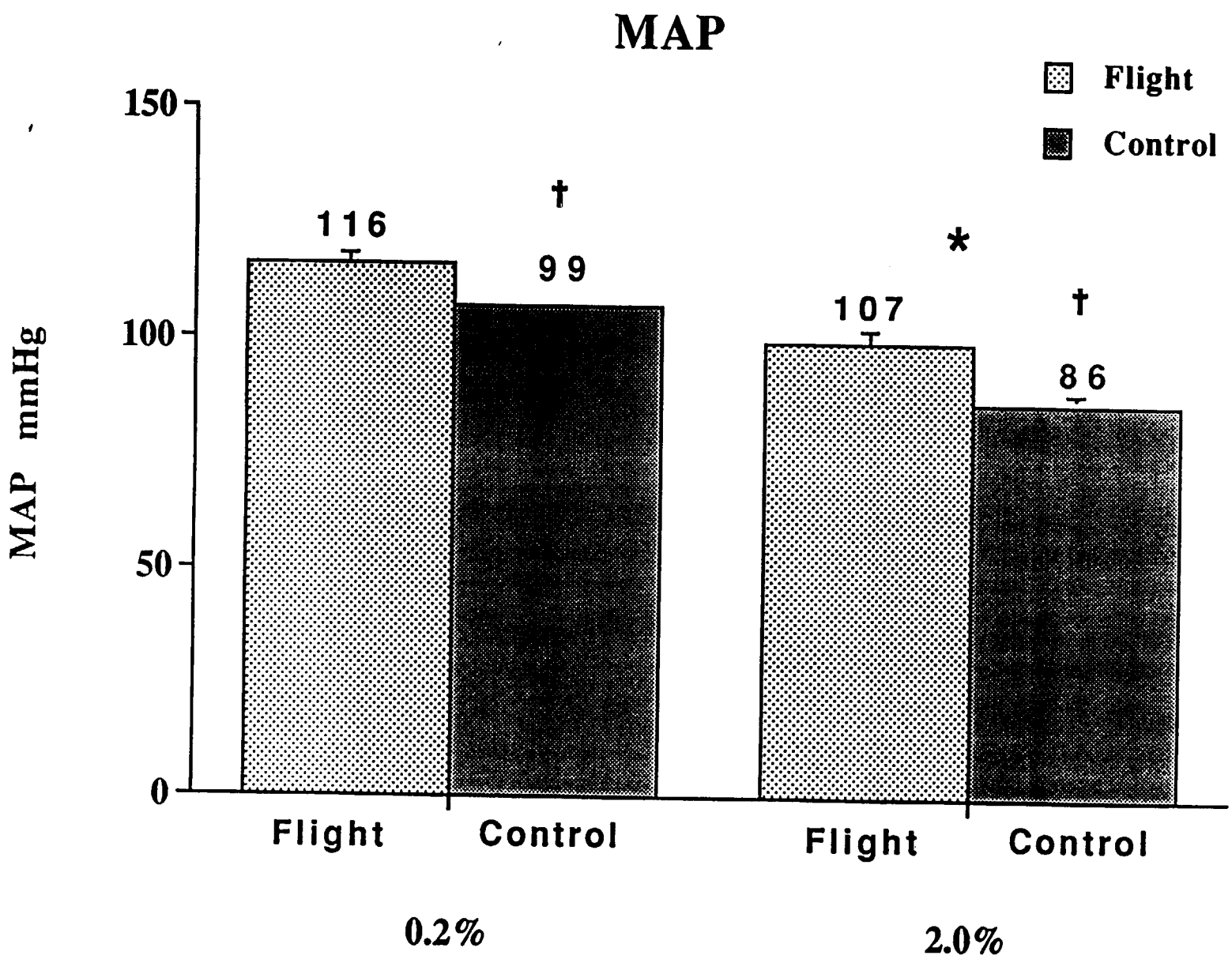
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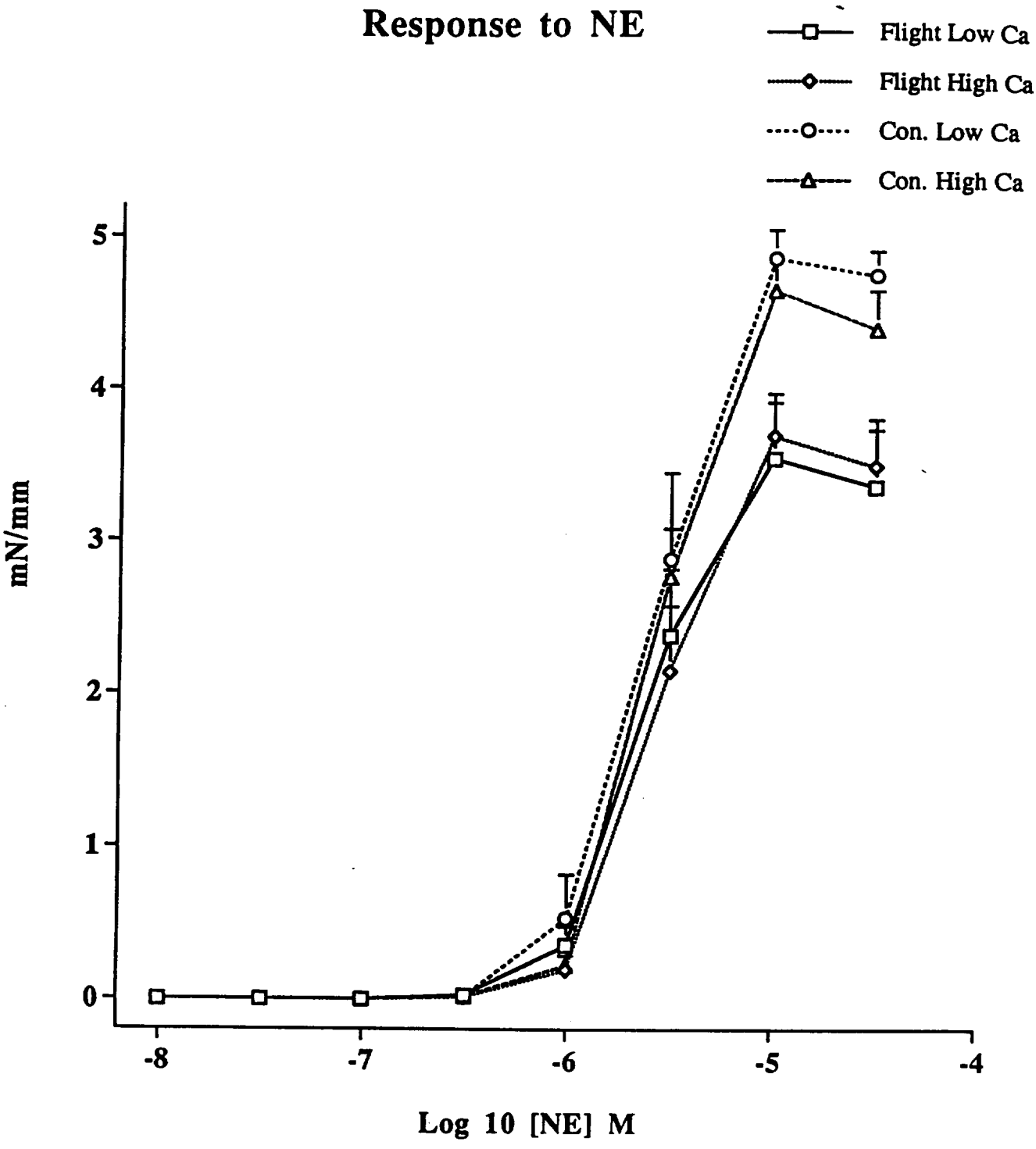
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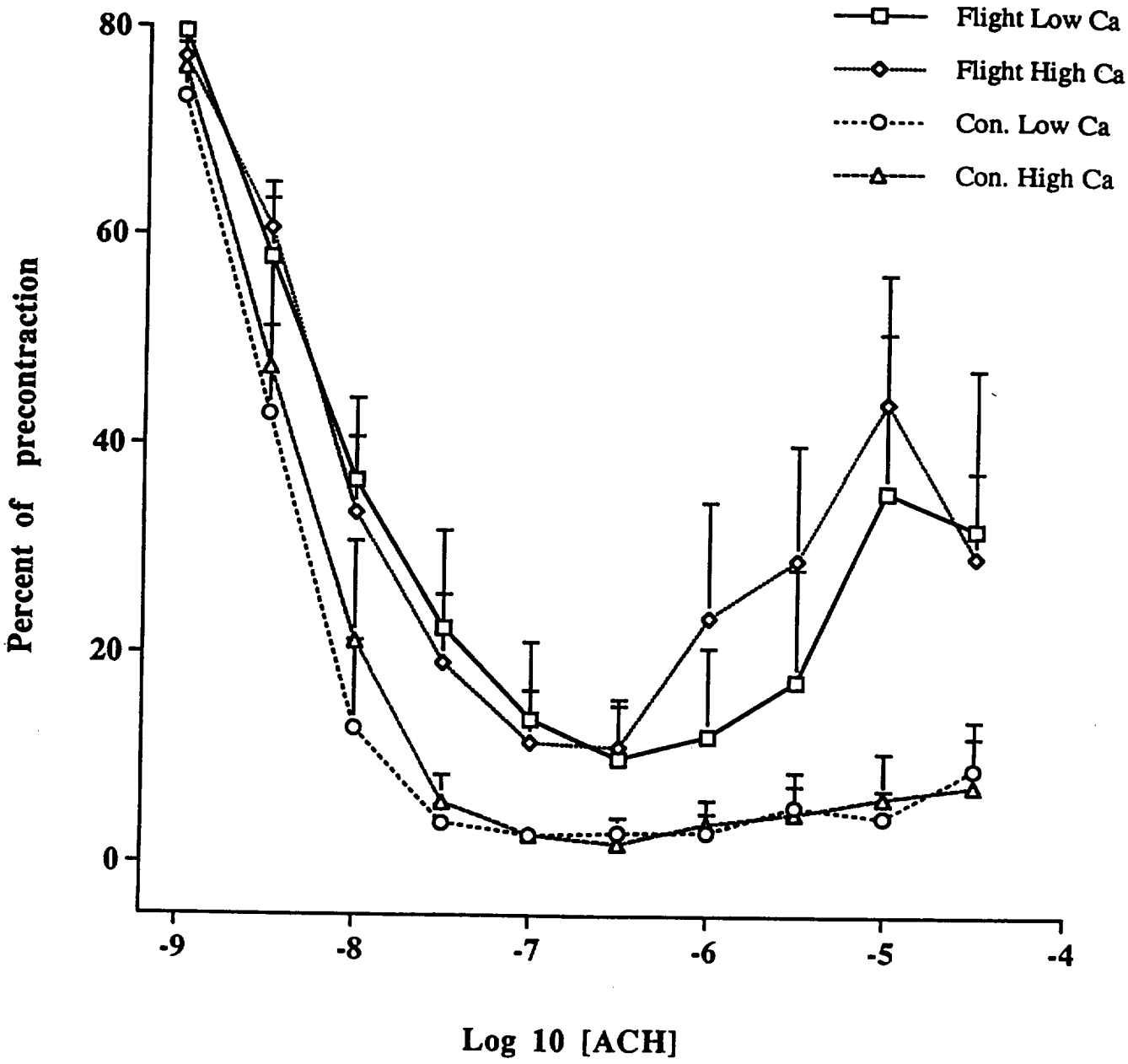
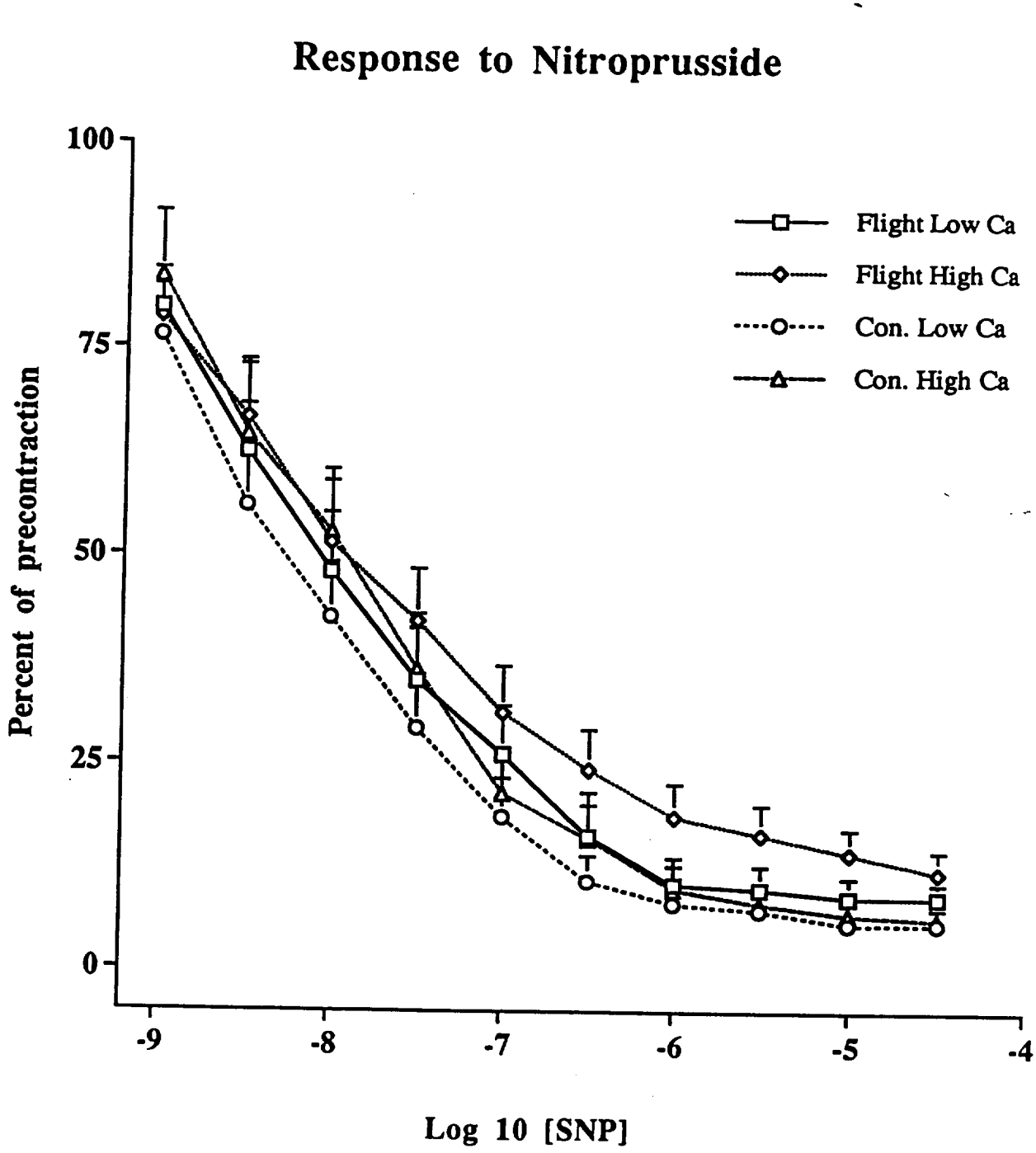
Response to ACH

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Flight High Ca
Con. Low Ca
Con. High Ca

Response to Calcium