Microcirculatory Responses in the Bat Wing to Glucagon with and without Barbiturate Anesthesia[1] (36515)

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(Introduced by D. K. Meyer)

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The general cardiovascular response to glucagon administration has been described by numerous investigators. A majority of these reports have been concerned with data obtained from humans with various stages of cardiovascular disease, either during diagnostic catheterization or during the recovery period after cardiac surgery (1–4). Experimentation in dogs and cats with glucagon has also been reported (5–7). A consensus of these investigations indicates that glucagon produces positive inotropic and chronotropic effects on the heart. Systemic blood pressure and peripheral resistance have been reported to increase (2, 10), decrease (7) or remain unchanged (1, 4). These responses are not dependent upon beta-receptor mechanisms as beta blockade by propranolol does not alter the observed responses (7, 11). There are studies which suggest that the cardiovascular effects of glucagon are potentiated in dogs and cats (7, 8) by, or reversed in man (1) and dogs (5) by the effects of barbiturate pretreatment.

The confusion about the peripheral responses to glucagon might be explained in part by the great diversity of protocols and patient subjects which are necessitated by the nature of clinical research. Alternatively, this confusion might be the result of indirect measurements since no data have been obtained by direct observation of the microvasculature before and after glucagon administration. The present study was designed to quantitate directly the mammalian microvascular response in subcutaneous tissue to glucagon with and without barbiturate pretreatment.

Methods. These studies utilized closed-circuit television microscopy to quantitate the microvascular responses in the bat wing (15). In essence, an unanesthetized bat (7–14 g *Myotis sodalis*) was restrained with wings extended on a microscope stage. An image of a subcutaneous small artery (35 to 45 μ) and vein (70 to 100 μ) was selected for display on a television monitor with a calibrated magnification of approximately 1000×. The diameters of a small artery and vein were measured at 30 sec intervals. As described previously (14), the raw data for each of these variables were smoothed by a five-point digital computer filter and normalized to give data as percentage of mean control values for each experiment. Subsequently, the mean and standard error of mean (SEM) were calculated for each point in time to provide the graphs presented here. Data at 10 min after glucagon injection were compared to the data gathered during the control period by group t tests.

One experiment was performed on each of 23 animals. The protocol for each experiment consisted of: (a) animal preparation and microscopic selection of comparable vessels according to landmarks in wing structure; (b) a waiting period of at least 40 min in a darkened room to achieve a quiet condition; (c) an intraperitoneal injection of sodium pentobarbital, thiopental, or saline; (d) a 50 min

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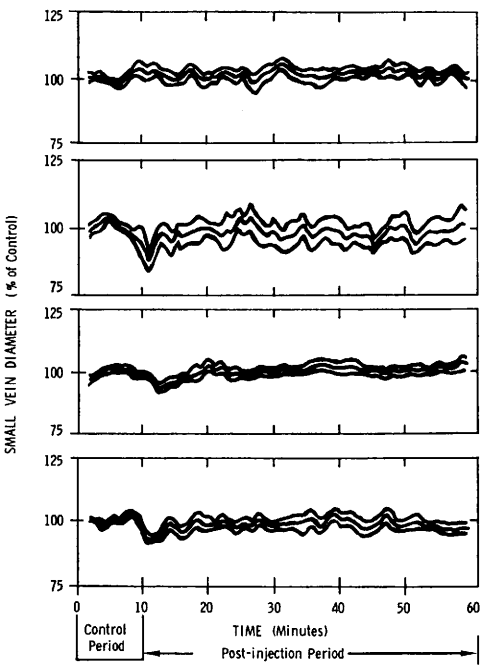

FIG. 1. Small vein response to glucagon with and without barbiturate pretreatment. (abscissa) Time in minutes with 0 and 10 min corresponding to the start of the control and postinjection periods, respectively. (ordinates from top to bottom) The small vein diameters for glucagon (130 mg/kg), for glucagon (130 mg/kg) after pretreatment with thiopental (20 mg/kg), for glucagon (130 mg/kg) after thiopental (50 mg/kg), and for glucagon (210 mg/kg) after thiopental (50 mg/kg) are expressed as percentage of their respective mean control values during the first 10 min. Ordinate values greater than 100% represent vasodilation while values less than 100% indicate vasoconstriction. The upper and lower tracings in each panel are the average data ± the SEM.

waiting period; (e) a 10 min control period for data collection; (f) an intraperitoneal injection of glucagon; and (g) a 50 min postinjection period for collection of data. The animal was observed for spontaneous head movements and was tested for a biting reflex in response to tactile stimulation on the nose. The dosages were 50 mg/kg for pentobarbital, 20 or 50 mg/kg for thiopental and 130 or 210 mg/kg for glucagon in concentrations to give an injection volume of 10 μl/g for the anesthetics and 1.0 μl/g for glucagon.

Results. In Fig. 1, the average responses of the small veins to glucagon (130 mg/kg), to glucagon (130 mg/kg) after pretreatment with thiopental (20 mg/kg), to glucagon (130 mg/kg) after pentobarbital (50 mg/kg), and to glucagon (210 mg/kg) after thiopental (50 mg/kg) are given in the four panels, top to bottom, respectively. Similarly, the average responses of the small arteries to the same drug combinations are given in Fig. 2. The missing data at 45 min is the result of a temporary loss of the arterial diameter data during one experiment (top panel). Data on the ordinate are presented as percentage of their mean value during the 10 min control period. Time of observation appears on the abscissa with glucagon administration occurring at 10 minutes. In each panel, the middle tracing is the mean curve for all animals in one of the four experimental groups; the upper and lower tracings correspond to

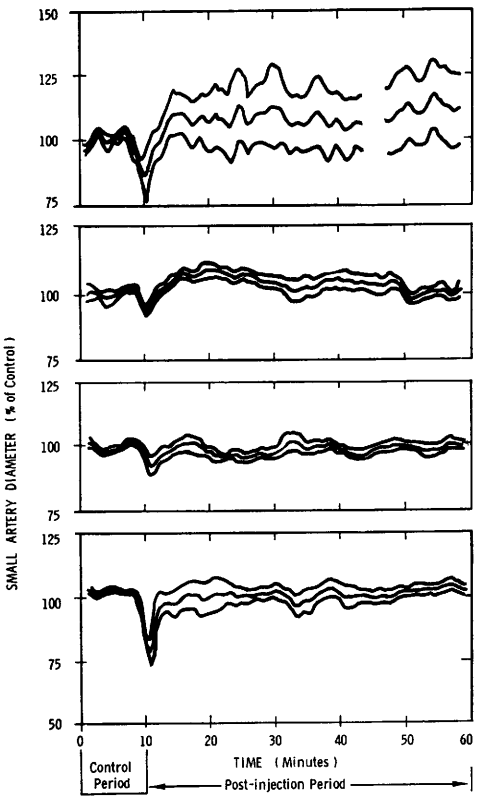

FIG. 2. Small artery response to glucagon with and without barbiturate pretreatment. The drug combinations and protocols are the same as those presented in Fig. 1.

the mean ± SEM.

Other than the transient constriction at the time of intraperitoneal injection of glucagon (10 min mark along abscissa in Fig. 1), there were no statistically significant responses of the small veins to glucagon with or without barbiturate pretreatment. Ten minutes after glucagon without barbiturate pretreatment (20 min mark on the abscissa, top panel in Fig. 2), the small arteries appeared to dilate to 108% ± 10% but this could not be demonstrated statistically ($p > .05$). With thiopental (20 mg/kg) pretreatment, the small arteries significantly dilated to 109% ± 2.5% ($p < .02$) at 10 min after glucagon (Fig. 2, second panel). In the lower two panels glucagon (130 mg/kg) following pentobarbital (50 mg/kg) and glucagon (210 mg/kg) following thiopental (50 mg/kg) did not produce any significant change in small artery diameters. As reported previously (12), pentobarbital (50 mg/kg) and thiopental (50 mg/kg) appeared to anesthetize the animals since they did not have spontaneous head movements nor a biting reflex to tactile stimulation on the nose. Thiopental (20 mg/kg) produced moderate sedation without hypnosis according to these qualitative criteria of anesthesia.

Discussion. The data presented in Fig. 1 indicate that the subcutaneous veins do not respond to glucagon, with or without pretreatment by barbiturates. This is not consistent with the hypothesis suggested by Williams *et al.* (13). They suggested that glucagon might have ". . . an effect on capacitance vessels and (effect) an increase in venous return. . . ." It is, however, possible that the veins in muscle tissue respond to perform this function.

In awake bats (Fig. 2, second panel), the arterial data suggest that a dilatation of about 10% occurs at 10 min with a return to control values occurring in about 30 min. This time course for the response to glucagon corresponds in general to that reported in the literature. It should be noted that vascular resistance is proportional to the fourth power of the vessel diameter and that a 10% change in arterial diameter would correspond to a change in vascular resistance approaching 50%. Therefore, while changes in vessel diameter might be slight, the change in vascular resistance could be significant. If other organ systems respond in a similar manner, the cumulative effect upon total peripheral resistance would be considerable. For example, Bache *et al.* (16) report reductions in total vascular resistance of 35 to 44% in unanesthetized dogs. Similarly, Puri and Bing (17) report reductions in peripheral resistance of 44% in dogs lightly anesthetized with pentobarbital, 25 mg/kg.

In previous studies (14, 15) we observed that small arteries and veins constricted or dilated independently of each other with changes in diameter as large as 50% of control values. Thus, the dilatation of small arteries observed in this study does not represent the maximum dilatation which is possible. Both arteries and veins in other microcirculatory preparations are innervated and possess beta receptors. Therefore, the lack of small vein dilatation in conjunction with small artery dilatation in this study suggests that the peripheral vascular response to glucagon is not mediated through beta-receptor mechanisms.

The lack of an arterial response in the anesthetized animals (Fig. 2, bottom two panels) agrees with the findings of Farah and Tuttle (5). They anesthetized intact dogs with pentobarbital (30 mg/kg) and observed no changes in peripheral resistance after glucagon. In particular, these observations do not give any indication that the glucagon response is potentiated by barbiturates as suggested in a recent review by Kones and Phillips (8).

Summary. The response of the subcutaneous small arteries (35–45 μ) and veins (70–100 μ) to glucagon, with and without barbiturate anesthesia, was quantitated by direct observations in the bat wing. No statistically significant venular response was observed in either the awake or anesthetized bat. An apparent, but not significant, increase in arterial diameter to 108 ± 10% ($p > .05$) of control values was observed 10 min after intraperitoneal injection of glucagon (130 mg/

kg) without barbiturate anesthesia. With thiopental (20 mg/kg) pretreatment, the small arteries significantly dilated to 100 $\pm$ 2.5% ($p < .02$) at 10 min after glucagon with a return to control values within 30 min. No changes in arterial diameters were observed with glucagon after pretreatment with a higher dose (50 mg/kg) of either pentobarbital or thiopental. It is concluded that glucagon could produce significant reductions in peripheral resistance if other organ systems respond in a manner which is similar to subcutaneous tissue.

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