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Effect of adrenaline on the glucose uptake by the canine hindlimb

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Summary
The effect of adrenaline on the glucose uptake by a non-exercising hindlimb was studied in fasted anaesthetized dog. Glucose uptake (mg/min) was calculated as the product of the venous blood flow and anterio-venous glucose difference (A - V). Although, adrenaline caused significant increase in venous blood flow, it however, reducued significantly the (A - V) glucose level and the glucose uptake by the hindlimb. The adrenaline effects were however, abolished by pretreating the animal with propranolol, a β-adrenergic blocker.It was therefore concluded that the skeletal muscle of the canine hindlimb is not involved in glucose homeostasis during adrenaline hyperglycemia.

Keywords: *Adrenaline, glucose uptake, propranolol, prazosin, hindlimb, dog.*

Résumé
L'effet de l'adrenaline sur la prise du glucose par un member superieur qui ne subit pas d'exercise a ete etudie chey un chien a jeune anaesthezie. La prise du glucose (mg/min0 a ete calcule comme le produit de la coulee veineuse du sang et la difference anterior-veineu se da glucose (A-V). Bien que l'adrenaline causait une augmentation significative dans la circulation veineuse du sang, cela reduisant cependant, de facon significative le (A-V) nivean du glucose et la consommation du glucose par le member superieur. L'effet de l'adrenaline etait cependant aboli par un pre-trailement de l'animal au propranolol, un adrenergique B. Il a ete alors conclu que le muscle du squelette de la canine du member superieur n'est pas inclu dans l'homestasie du glucose lors de l'hyperglycemie de l'adrenaline.

Introduction

The skeletal muscle is the major site of disposal of the excess postprandial plasma glucose [1,2,3]. It is also estimated to account for approximately 90% of glucose uptake during euglycaemic hyperinsulinaemia in humans [4]. Apart from this, the skeletal muscle accounts for about 20 to 30% of oxygen consumption at rest and as much as 90% during exercise [5,6]. Therefore, the importance of the skeletal muscle in glucose metabolism is not in doubt.

Several studies [7,8,9,10] have reported that catecholamines decreased glucose utilization by the skeletal muscles. However, Chiasson *et al.* [11] in an *in vivo* experiment reported that adrenaline exhibits a stimulatory effect on the glucose uptake of the perfused rat hindlimb. We did not find any publication in the literature on the effect of adrenaline on the resting canine hindlimb skeletal muscles.

Previous studies in canines [12,13,14,15,16] have established firmly that the gastrointestinal tract (g.i.t) played a role in glucose homeostasis. During induced hyperlycemia irrespective of its cause, the gut increases its glucose uptake and when there is hypoglycaemia the gut actually releases glucose into the circulation [15]. The question is, does the canine hindimb, which is mainly skeletal muscle and which accounts for 46% of the dog's body mass [17], also respond with increased uptake of glucose when challenged with adrenaline as in the gut?. The present study was therefore undertaken to investigate the effect of adrenaline on the glucose uptake by a non-exercising canine hindlimb.

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Materials and methods

Male mongrel dogs weighing 11-15kg were used for the study. Each animal was fasted for 18-24hr before the start of an experiment. Anaesthesia was induced by i.v. sodium pentobarbitone, 30mg/kg. Light anaesthesia was maintained with supplemental doses of i.v. sodium pentobarbitone as necessary. The trachea was incubated using a Y-piece cannula and the animal was allowed to breathe room air (temp. 25°C) spontaneously.

The right femoral vein and artery were cannulated. The cannula in the right femoral vein was moved into an extra-corporeal position and a non-crushing clamp was applied to its free end. The left femoral vein was also cannulated for the administration of drug. Sodium heparin, 300 units per kg was administered i.v. to prevent blood clotting.

Experimental procedure

Following surgery, a period of 60minutes was allowed for stabilization in all animals. The blood flow to the hindlimb was measured by timed collection of the blood from the right femoral vein as previously described [14]. Arterial and venous blood samples for glucose estimation were obtained from the cannula placed in the right femoral artery and vein respectively. After stabilization, basal measurements of the femoral venous blood flow, arterial and venous glucose levels were made. The experiments were carried out in four groups:

Untreated group (adrenaline only) (Group I)

Eight dogs were studied. After basal recording of blood flow and collection of arterial and venous blood samples for glucose estimation (0.05ml per sample) adrenaline, 3mg/kg was given i.n. as a bolus injection. The blood flow, arterial and venous blood glucose concentrations were measured at 0min, 5min, 10min, 15min, 20min, 25min, 30min, 45min, 60min, 75min and 90min during the post-injection observation period.

Pretreatment with propranolol (Group II)

Four dogs were first injected with propranolol before adrenaline injection. Each animal was given i.v. injection of propranolol, 0.5mg/kg. After forty minutes, basal recording of the femoral venous blood flow and sample collection for arterial and venous blood glucose were carried out and adrenaline, 3mg/kg was given as a bolus injection. The venous blood flow and the arterial and venous blood glucose concentrations were measured at intervals as in the untreated group.

Pretreatment with prazosin (Group III)

Four dogs were first given prazosin before adrenaline injection. Each dog was injected i.v. with prazosin, 0.2mg/kg. Forty minutes was allowed for the drug to take effect. Thereafter, basal measurements of the venous blood flow and arterial and

venous blood glucose were made. Adrenaline, 3mg/kg was then given as bolus injection. The blood flow and arterial and venous blood glucose concentrations were similarly monitored at intervals as in the untreated group.

Normal saline injection (control) (Group IV)
Four dogs were given bolus injections of 0.9% saline in similar volumes as the adrenaline injections after basal recording of the venous blood flow and arterial and venous blood glucose concentrations. The same parameters were similarly monitored during the 90min post-injection observation period.

Blood glucose measurement
Blood glucose was determined by the glucose oxidase method as modified by Trinder (18). Glucose uptake (mg/min) was calculated as the product of the arterio-venous glucose difference (A-V) and the femoral blood flow per minute.

All values given are the mean ± SEM of the variables measured. Significance was assessed by the student's t-test for two means of independent variables. P values of 0.05 or less were taken as statistically significant.

Results
The results are shown in Tables 1 and 2 Figures 1 and 2

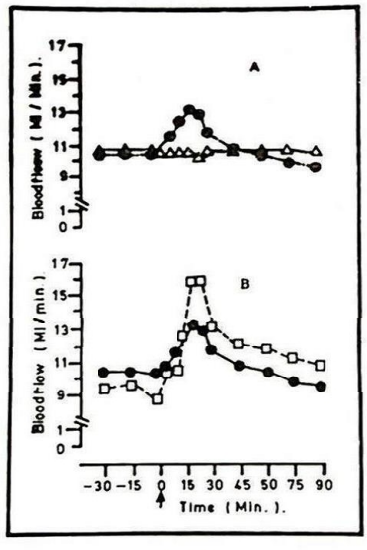

Fig.1:Effect of Adrenaline on the blood flow in (A), untreated (•-•) and propranolol-treated (-) dogs; (B) untreated (•-•) and prazosin-treated
(-) dogs

Effect of adrenaline on blood glucose, blood flow and glucose uptake
The effects of adrenaline on the arterial and venous blood glucose levels are shown in table 1. Adrenaline increased the arterial glucose level from a basal value of 86.4 + 6.3mg/dl to a maximum value of 125.6+ 5.7mg/dl twnty minutes post-injection. These increase were significant. Table 2 shows the effect of adrenaline on the arterio-venous glucose difference, (A-V). The (A-V) glucose decrease from a basal value of 11.3= 0.9mg/dl to a minimum value of 5.4 + 0.4mg/dl twenty minutes post-injection. In other words, the glucose extraction by the hindlimb decreased significantly.

Figure 1 shows the effect of adrenaline on the venous blood flow of the hindlimb. Adrenaline caused a significant increase in the blood flow from a basal value of 10.4 + 0.1 ml/min to a peak value of 13.2 + 0.2ml/min twenty minutes post-injection. Figure 2 shows that adrenaline produced a significant decrease in the glucose uptake by the hundlimb. Infact, the glucose uptake decreased from a basal level of 117.9 + 9.8 mg/min to a minimum value of 71.9 + 6.3 mg/min twenty minutes post-injection. This represents a 39% decrease from the basal glucose uptake value.

Effect of beta adrenergic blockade
The effects of pretreatment with propranolol on adrenaline-induced hyperglycemia are shown in table 1. In the group pretreated with propranolol, the arterial blood group increased at the peak of response to a higher value of 138.5+_ 11.2 mg/dl twenty minutes post-injection. Propranolol, however did not produce any significant effect on adrenaline-induced rise in venous blood glucose level. Table 2 shows that propranolol caused a slight but insignificant increase in (A-V) glucose induced by adrenaline. In other words, Propranolol abolished the adrenaline-induced decrease in glucose extraction by the hindlimb. Propranolol also abolished the increase in blood flow caused by adrenaline (figure 1).

Figure 2 shows that pretreatment of the animal with propranolol abolished the decrease in glucose uptake caused by adrenaline in the untreated animal. Indeed, propranolol produced a significant increase in the hindlimb glucose uptake. There was an increase of about 26% above the basal value.

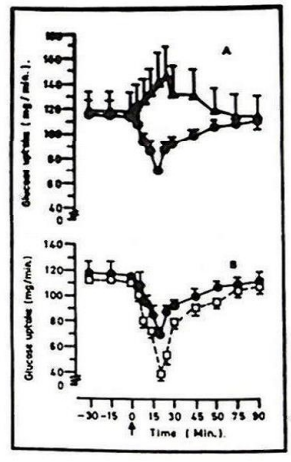

Fig. 2: Effect of Adrenaline on the glucose in (A), untreated (•-•) and propranolol-treated (-) dogs; (B) untreated (•-•) and prazosin-treated (-) dogs.

Effect of alpha adrenergic blockade
Prazosin significantly reduced the peaks of adrenaline-induced increase in arterial and venous glucose levels (Table 1).

Table 2 shows that prazosin caused a further decrease in arterio-venous glucose difference (A-V) produced by adrenaline. Again, prazosin potentiated the effect of adrenaline on blood flow (figure 1). The peak value of adrenaline-induced increase in blood flow was 15.9 ± 0.6ml/min after prazosin pretreatment. This represents 62% rise above the basal value. Figure 2 shows that prazosin produced a further decrease in adrenaline-induced effect on glucose uptake by the hindlimb.

Table 1: Effect of adrenaline on the arterial andvenous plasma glucose concentration

		Time (min.)											
		-30a	-15a	0	5	10	15	20	25	30	45	60	75	90
		Concentration (mg)/dl)												
Treatment														
Untreated (8)	Arterial	87.3 ±6.1	87.3 ±6.0	86.4 ±7.1	97.1* ±4.7	102.6* ±4.1	121.5* ±5.9	131.0* ±6.1	127.1 ±4.1	*1 20.1 ±4.5	*108.2* ±7.2	96.7± 6.3	90.3 ±6.0	86.5 ±6.5
	Venous	76.0 ±5.5	76.0 ±5.3	75.1 ±6.3	86.7* ±4.0	94.3* ±3.9	114.4 ±5.4	125.6* ±5.7	120.3* ±4.1	120.0* ±4.3	99.0* ±7.2	86.3 6. 0	78.9 ±5.7	74.6 ±6.2
Propra-Nolol Pretreated (4)	Arterial	89.6 ±6.4	89.6 ±6.1	89.7 ±6.5	101.3* ±5.1	110.6* ±11.5	130.4* ±12.2	138.5* ±11.2	131.6* ±3.7	126.2* ± 4.3	127.0* ±7.5	104.6 ±5.9	93.4* ±7.8	91.5 ±4.6
	Venous	78.6 ±5.1	78.6 ±4.7	78.7 ±5.1	89.1* ±4.0	98.5* ±10.9	117.5 ±11.9	124.8* ±11.1	117.2* ±3.1	113.7* ± 4.3	114.8* ±7.5	93.4* ±5.9	83.0 ±7.8	80.4 ±4.6
Prazosin Pretreated (4	Arterial	93 2 ±4.2	92.8 ±4.1	95.5 ±3.4	101.3 ±4.6	113.3 ±2.2	116.0 ±2.0	122.2* ±1.8	116.7 ±4.8	108.4 ±1.2	97.5 ±2.0	81.6 ±3.3	84.7 ±0.3	83.2 ±1.0
	Venous	81.1 ±3.9	81.1 ±3.8	82.8 ±3.5	91.1 ±4.5	105.1 ±2.8	110.6 ±2.1	119.6 ±1.8	113.2 ±5.2	102.0 ±1.7	89.4 ±2.0	72.8 ±4.3	74.7 ±1.1	72.5 ±1.9

*Means SEM Basal values; * Significant (P<0.05); "n" is in brackets.*

Table 2: Effect of adrenaline on the arterio-venous glucose difference in untreated and pretreated dogs

	TIME (min.)												
	-30*	-15*	0	5	10	15	20	25	30	45	60	75	90
Treatments	**Concentration (mg/dl**												
Untreated (8)	11.4 ±0.9	11.3 ±0.9	11.3 ±0.9	10.4 ±1.0	8.3* ± 0.5	7.1* ±0.5	5.4* ±0.5	6.8* ± 0.4	8.1 ± 0.4	9.2 ±0.2	10.5 ± 0.5	11.3 ± 0.5	11.9 ± 0.5
Propranolol pretreated (4)	11.0 ±1.3	11.0 ±1.4	11.0 ±1.4	12.2 ±1.1	12.1 ± 0.6	13.0 ± 0.7	13.7 ± 0.8	14.4 ±1.1	12.5 ±1.1	12.2 ±1.3	11.2 ±1.3	10.8 ±1.5	11.1 ±1.8
Prazosin pretreated (4)	12.0 ±0.3	11.8 ±0.4	12.7 ±0.4	10.1 ±1.0	8.1 ±1.1	5.4* ±0.1	2.6* s± 0.1	3.5* ±0.5	6.5* ±0.8	8.2* ± 0.8	8.8 ±1.2	9.9 ±1.1	10.7 ±1.3

*Mean SEM; Basal value; *significant (P<0.05); "n" is in brackets*

Effect of normal saline

0.9% saline had no effect on the arterial and venous blood glucose levels. It also had no effect on blood flow and hindlimb glucose uptake. The mean resting blood flow of the hindlimb was 117.9± 9.8mg/min.

Discussion

The increases in arterial and venous blood glucose observed in this study as a result of adrenaline injection is consistent with the well-known pharmacological effects of adrenaline [19, 20,21]. The increase in blood flow observed in this study when adrenaline was administered also agrees with previous reports [12, 15, and 22]. The mechanisms by which adrenaline increased blood glucose [23] and blood flow [24] are well documented.

The significant decrease in glucose uptake by the hindlimb following adrenaline injection observed in the present study agrees with some of the previous reports [7,8,9,25,26]. Although, adrenaline increased the flow of blood to the hindlimb, the decrease in hindlimb glucose uptake in this study also correspond in timing with the decrease in arterio-venous glucose difference. In other words, there was actually a significant reduction in glucose extraction by the canine hindlimb. Adrenaline has been reported to inhibit glycogenesis in tissue [27] and suppress glucose clearance [28].

The reversal of the adrenaline-induced decrease in hindlimb glucose uptake by propranolol in this study suggests the involvement of B- adrenergic receptors in the mechanism by which adrenaline reduced hindlimb glucose uptake. Failure of prazosin to alter the hindlimbs glucose uptake shows that alpha-adrenergic receptors are not involved in the adrenaline induced decrease in hindlimb glucose uptake.

In conclusion, the present study has shown that during adrenaline-induced hyperglycemia, the glucose uptake by the hindlimb decreased significantly. And that this decrease in glucose uptake was mediated by B-adrenergic receptors. The

results also show that the skeletal muscle of the canine hindlimb is not involved in glucose homeostasis during adrenaline-induced hyperglycemia.

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