

Multiphasic Insulin Response to Arginine¹ (35681)

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Although arginine is known to be an effective stimulus for insulin release, little is known of the mechanisms by which release occurs. Recently, Porte and Pupo (1) and Grodsky *et al.* (2) while studying glucose-stimulated insulin release, have pointed to the multiple phase configuration of plasma insulin curves. They suggest that the phases are due to different insulin release mechanisms and propose a multiple pool or compartmental model for insulin secretion.

In this study, we investigated the immediate and protracted insulin response to both rapid and prolonged arginine administration to determine if an arginine stimulus produces a multiphase insulin curve.

Methods and Materials. Mongrel puppies, 24 to 36 days of age and weighing 2.0 to 3.8 kg, were allowed only water 6 to 8 hr prior to the study. They were lightly anesthetized with intramuscular morphine sulfate (2 mg/kg) and intraperitoneal pentobarbital (15 mg/kg). The puppies were then intubated and ventilated with air on a small animal respirator. Under 2% lidocaine hydrochloride local anesthesia, the femoral vessels were isolated and polyethylene catheters were placed in the artery and the vein. The catheters were filled with saline containing no anticoagulants. To facilitate ventilation and to prevent muscle activity, a constant infusion of succinylcholine hydrochloride was maintained via one of the venous catheters. Ambient temperature was maintained between 27 and 29° with external lamps to

avoid cold stress (3).

Approximately 30 min after surgery was completed, and when arterial pH, pO₂ and pCO₂ were stable in a normal range (pH 7.3 to 7.4; pO₂ > 70 mm Hg; pCO₂, 32–38 mm Hg), control observations were initiated. At the completion of the control period which also included arterial samples for plasma immunoreactive insulin (IRI) and glucose, *l*-arginine, adjusted to pH 7.3–7.4, was administered intravenously as a pulse (injection time less than 5 sec, in a dose of 0.1 to 0.5 g/kg) to eight puppies, and by constant infusion (1.5 g/kg/hr) to five puppies for 1 hr. Corrections in respirator parameters and intravenous sodium bicarbonate were used to maintain normal ranges of blood gases and pH during the control and study periods. Arterial samples were centrifuged under refrigeration; and the plasma was frozen until analyzed for IRI (4, 5) and glucose (6).

Results and Discussion. In our young dogs, an arginine pulse caused a rapid elevation of plasma IRI levels to 693% ± 121 of mean control levels (mean ± SE). The peak occurred in 1 min and then quickly decayed. Circulating IRI levels returned to control values within 30 min of the peak (Fig. 1).

With 1-hr infusions of arginine, an early rise and distinct fall in plasma IRI was again noted despite the continuing infusion of arginine. However, the plasma IRI did not return completely to base line but remained elevated above control levels throughout the 60 min of arginine administration (Fig. 1).

To determine if the sustained elevation of IRI levels was significant, the two groups of IRI data (Fig. 1) were tested with Student's *t* test at each time interval. Values for pulse and infusion groups were significantly different at 1, 10, 15, 30, and 60 min (Table I). To minimize variability effects the two

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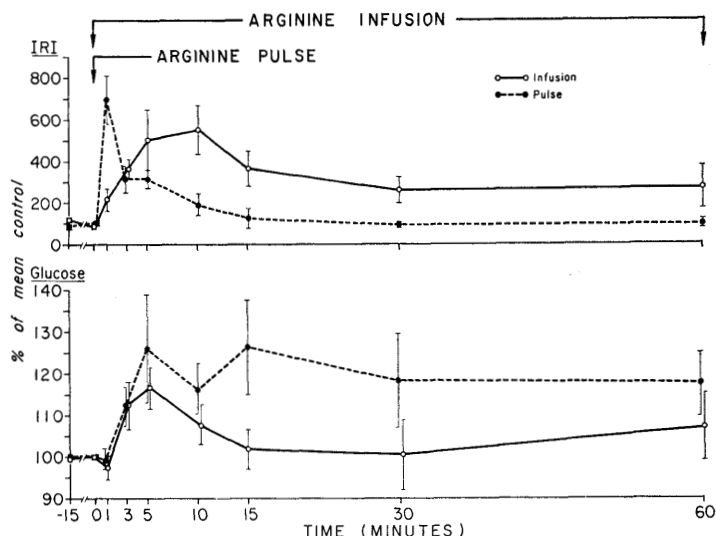


FIG. 1. Response of plasma immunoreactive insulin (IRI) and plasma glucose to an arginine pulse (arrow) in eight puppies and an arginine infusion in five puppies. Data points represent mean. Vertical bars represent \pm SE.

groups of IRI data (Fig. 1) were converted to \log_{10} and again compared with Student's t test (7). Differences at 1, 10, 15, 30, and 60 min continued to be significant (Table I).

The configuration of plasma glucose curves in the puppies were generally similar to that of plasma IRI, but the changes were considerably smaller, more gradual and often delayed in onset when compared with the plasma IRI (Fig. 1).

Changes in plasma glucose value were not significantly different in pulse and infusion

TABLE I. Comparison of Infusion and Pulse IRI Values.

Samples (min)	t^a	p^a	t^b	p^b
Control	0.019	>0.50	0.042	>0.50
1	2.939	<0.02	2.403	<0.50
3	0.695	>0.50	0.963	>0.20
5	1.512	>0.10	1.988	>0.05
10	3.164	<0.01	3.461	<0.01
15	2.591	<0.05	3.259	<0.01
30	3.320	<0.01	4.023	<0.01
60	2.201	0.05	2.834	<0.02

^a t test and p values on IRI data as presented in Fig. 1.

^b t test and p values on IRI data after logarithmic transformation to minimize variability.

groups except at the 15-min sample ($t = 2.348$, $p < 0.05$).

The data indicate that the plasma insulin curve in puppies has distinct phases; the exact configuration being dependent on the duration of the arginine administration (Fig. 1). This suggests that arginine, as well as glucose, stimulates insulin release by more than one mechanism.

As when glucose is given rapidly intravenously (1), an arginine pulse causes large increases in plasma insulin almost immediately, implying that the acute phase is not dependent on synthesis or other time-consuming processes, but on a source immediately available. Although insulin from the same acute source appears to be released during the initial phase of continuous infusion, with the sustained arginine stimulus, there is continued insulin output. This sustained second phase seems dependent upon insulin produced by a different mechanism, such as synthesis, or a more remote storage source requiring a longer interval for transfer of insulin to the circulation. However, our data only permits speculation on these etiologic possibilities.

Although arginine has been widely used in evaluating insulin secretion and others have documented parts of the insulin curve

(8–10), the early peak and subsequent decline in the insulin response to arginine infusion have not been appreciated. The reason in part appears to be a lack of early sampling time in most prior studies. Responses observed here indicate clearly the multiphasic nature of the insulin response and point to the critical importance of frequent early sampling when studying insulin secretion *in vivo*.

Summary. Although arginine is known to release insulin, the mechanism is poorly understood. Recently, it has been suggested that glucose-stimulated insulin release involves multiple mechanisms or pools. To determine if similar mechanisms are involved in arginine-induced insulin release, the immediate and more prolonged effects of arginine infusion were investigated in puppies. With intravenous arginine pulses, plasma immunoreactive insulin (IRI) rose dramatically ($693\% \pm 121$ of mean control), returning to base line levels within 30 min. With arginine infusions, an initial transient surge of IRI was again noted, but in contrast to the pulse, levels remained elevated throughout the infusion period. The two phases of insulin release observed during prolonged arginine infusion suggest that arginine, like glucose, stimulates insulin release by more than one

mechanism.

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