# The Effect of Hypoxia and Epinephrine Infusion on Arginine Induced Insulin Release[1] (37575)

JAMES W. FRENCH, DANIEL PORTE, JR., AND DAVID BAUM

*Department of Pediatrics, Stanford Medical School, Stanford, CA 94305 and Department of Medicine, University of Washington Medical School, Seattle, Washington 98105*

Arginine and glucose are known to be potent stimuli of insulin secretion under aerobic conditions. However, Baum and Porte (1) have recently shown that hypoxia inhibits glucose stimulated insulin release in the young dog. Because glucose and arginine are thought to stimulate insulin release by different mechanisms (2) the present study was designed to investigate the effect of hypoxia on arginine stimulated insulin release in the puppy. The effect of epinephrine infusion on arginine stimulated release was also examined.

*Methods and Materials.* Mongrel puppies 24 to 36 days of age and weighing 2 to 4 kg were allowed only water for 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 local anesthesia with 2% lidocaine hydrochloride, the femoral vessels were isolated and polyethylene catheters were placed in the artery and in 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 provided via one of the venous catheters. Ambient temperature was maintained between 27 and 29° to avoid cold stress (3). Approximately 30 min after surgery was completed, and when arterial pH, $PO_2$ and $PCO_2$ were stable in the normal range (pH 7.3 to 7.4; $PO_2 > 70$ mm Hg; $PCO_2$ 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 g/kg. Arterial blood samples were obtained 1, 3, 5, 10, 15 and 30 min after injection. After the control injections six puppies were subjected to an experimental period of hypoxia (arterial $PO_2$ 18–24 mm Hg) and 5 puppies to epinephrine infusion (0.5 μg/kg/min) for 60 min. Thirty minutes after the start of the experimental period, a second arginine pulse was administered and an identical chronological sequence of samples was obtained. Thus, each dog acted as its own control.

Total blood loss was estimated at less than 10% of total blood volume and each sample was replaced immediately with an equal volume of normal saline. Sodium bicarbonate was used to maintain the pH > 7.25 during hypoxic periods. Arterial samples were centrifuged under refrigeration and the plasma frozen until analyzed for IRI (4, 5) and glucose (6).

*Results. IRI.* During control periods, the intravenous arginine pulse stimulated a characteristic spike in IRI levels of short duration (Figs. 1 and 2). The mean change was 81.5 ± 11.1 (μU/ml ± SEM) in the hypoxia control group and 89.8 ± 16.0 in the epinephrine infusion control group. Base line levels were achieved within 10 min after the pulse. With the onset of hypoxia there was

[1] Supported in part by Public Health Service Grant HD-06775-02 from National Institute of Child Health and Human Development and Grant AM-08865 from National Institute of Arthritis and Metabolic Disease. Presented in part: 45th Scientific Sessions of the Amer. Heart Ass., Nov. 1972, Dallas, TX.

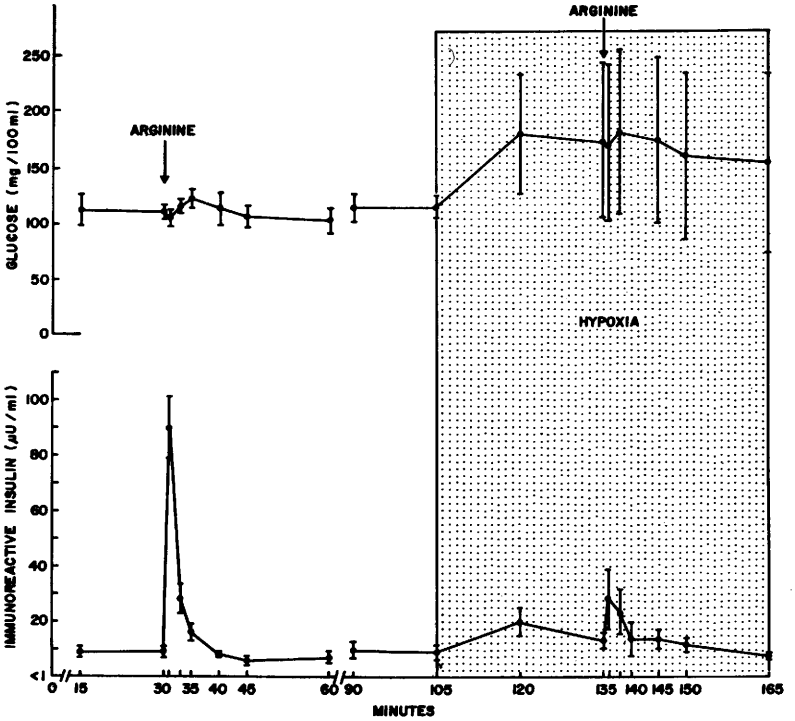


FIG. 1. Arginine pulse during control (air breathing) and hypoxic periods; mean ± SE.

an initial small rise in IRI with a return to base line prior to the arginine pulse (Fig. 1). Arginine caused a mean change of 20.2 ± 9.0 μU/ml in plasma IRI levels; an insignificant rise ($p > 0.10$). During the epinephrine infusion arginine pulses stimulated plasma IRI spikes which closely resembled the controls (mean change 98.4 ± 16.3 μU/ml) (Fig. 2). The plasma IRI responses to arginine during control and hypoxia periods were significantly different from each other (Student's $t$ test, $p > 0.02$, $t = 3.6$). However, the arginine induced response during the epinephrine infusion period was not significantly different from that obtained in the control period ($p > 0.5$, $t = 0.38$).

*Glucose.* During both control periods (hypoxia and epinephrine infusion) plasma glucose went up slightly as a result of the arginine pulse (Figs. 1 and 2). At the onset of both hypoxia and epinephrine infusion there was an elevation of plasma glucose, documenting a metabolic response to both stimuli.

*Discussion.* In a previous communication we have reported that an arginine pulse induced a rapid release of insulin in the young dog (7). This report demonstrates inhibition of that acute phase of insulin release during hypoxia.

Recent investigation of the insulin release control mechanism suggests that circulating catecholamines and the adrenergic receptors of the automonic system are of primary importance (8). Floyd and co-workers (9) have reported that arginine stimulation of insulin release depends on a balance of alpha and beta adrenergic receptor activity and that a dominant alpha activity is required for inhibition.

Alpha adrenergic receptor activity has also been proposed as part of the mechanism for the inhibition of glucose induced insulin release during hypoxia (8), a state associated with high circulating catecholamines (10). To examine the effect of elevated circulating catecholamines on arginine induced release, an epinephrine infusion was employed. The finding of continued insulin release during epinephrine administration is consistent with

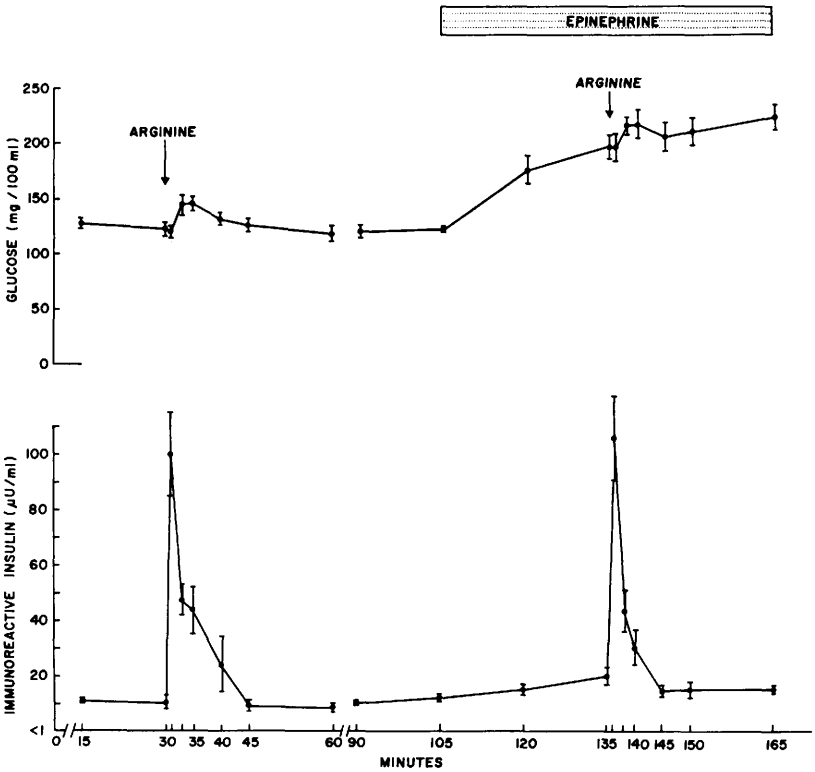


FIG. 2. Arginine pulse during control and epinephrine infusion; mean ± SE.

observations of other workers (8, 9, 11, 12). Our failure to demonstrate inhibition of arginine stimulated acute insulin release by means of epinephrine infusion suggests that either circulating catecholamines may not be a major factor in the inhibition seen during hypoxia or that epinephrine infusion in the puppy does not provide a sufficiently dominant alpha effect for inhibition.

*Summary.* The rapid phase insulin release induced by arginine in the puppy, was inhibited significantly by hypoxia, but not by epinephrine infusion. Based on these data we conclude that elevated epinephrine levels were not a major factor in the inhibition of the arginine effect caused by hypoxia. On the other hand, in the puppy, epinephrine may not produce sufficient alpha adrenergic stimulation to inhibit insulin release.

1. Baum, D., and Porte, D., Jr., Amer. J. Physiol. **222**, 695 (1972).

2. Efendic, S., Cerasi, E., and Luft, R., J. Clin. Endocrinol. **34**, 67 (1972).

3. Baum, D., and Porte, D., Jr., J. Clin. Endocrinol. Metab. **29**, 991 (1969).

4. Samols, E., and Bilkus, D., Proc. Soc. Exp. Biol. Med. **115**, 79 (1964).

5. Morgan, D., and Lazarow, A., Diabetes **12**, 115 (1963).

6. Moore, H. G., and Randle, P. J., Biochem. J. **93**, 66 (1964).

7. French, J. W., Baum, D., and Porte, D., Jr., Proc. Soc. Exp. Biol. Med. **137**, 858 (1971).

8. Porte, D., Jr., J. Clin. Invest. **46**, 86 (1967).

9. Floyd, J. C., Fajans, S. S., Pek, S., Knopf, R. F., Weissman, P., and Conn, J. W., J. Lab. Clin. **78**, 990 (1971).

10. Comline, R. S., and Silver, M., Brit. Med. Bull. **22**, (1966).

11. Rabinowitz, D., Merimee, T. J., Burgess, J. A., and Riggs, L., J. Clin. Endocrinol. **26**, 1170 (1966).

12. Efendic, S., Cerasi, E., and Luft, R., Metabolism **20**, 568 (1971).

Received April 5, 1973. P.S.E.B.M., 1973, Vol. 144.