Effects of Pentobarbital on Plasma Glucose and Free Fatty Acids in the Rat (36116)

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Animals are frequently anesthetized prior to blood collection or tissue sampling to expedite handling. Nembutal,[2] a drug containing sodium pentobarbital as the active anesthetic agent, is popular for such purposes.

In this laboratory, it became necessary to institute special bleeding procedures. Rats were anesthetized with Nembutal, the abdominal cavity was opened, and blood was removed by needle puncture of the aorta. By happenstance, it was found that plasma free fatty acids were lower and plasma glucose higher in the special treated rat than the values normally found in decapitated rats. It was not readily apparent whether the differences were related to the method of blood collection, animal variation, or to the anesthetic agent. The following experiments are a follow-up of these observations and describe changes that occurred in plasma glucose and free fatty acids as a result of Nembutal injection.

Methods. Male Sprague-Dawley rats (240–255 g) were fed *ad libitum* and allowed free access to water until initiation of the experiment. The rats were anesthetized by injection of 25 or 50 mg/kg of body weight of sodium pentobarbital (Nembutal) ip in a volume of approximately 0.25 ml. Nonanesthetized control rats were treated in one of three ways: (i) noninjected; (ii) injected with 1 ml/kg of body weight of 0.9% saline ip; or (iii) injected with 1 ml/kg of body wt of the Nembutal vehicle ip, which consisted of 10% ethanol, 20% propylene glycol, and 70% distilled water by volume and adjusted to pH 10.5 with NaOH. All injections were between 0900 and 0945 hr, and the rats were sacrificed at various time intervals up to 4 hr thereafter. Blood was collected in heparinized syringes via abdominal aorta puncture or in heparinized tubes after decapitation. All nonanesthetized control rats were decapitated. Plasma glucose was determined by the glucose oxidase method (1) and plasma free fatty acids (FFA) by the method of Trout *et al.* (2). The level of significance was $p<.05$ using a multiple t test. In the time course studies, the p value as determined by the t test was confirmed by the analysis of variance using the method of Scheffé (3).

Results. Fifteen minutes after the injection of saline or the Nembutal vehicle, plasma glucose significantly increased from a mean of 127 mg/100 ml in the noninjected control to 150 mg/100 ml in the saline- and vehicle-treated rats (Fig. 1 upper). The injection of pentobarbital (25 mg/kg of body wt) further increased plasma glucose to 160 mg/100 ml, a value significantly higher than that in the noninjected controls but not significantly different from the level in the saline- or vehicle-treated rats. However, when the dose of pentobarbital was increased to 50 mg/kg of body weight, plasma glucose rose to 197 mg/100 ml, which was significantly higher than the level found in saline, vehicle or 25 mg/kg of body weight pentobarbital-treated rats. Plasma obtained via aortic puncture after the injection of 50 mg of pentobarbital/kg of body weight had a glucose level of 180 mg/100 ml, which was comparable to the glucose level in decapitated rats which received the same dose of pentobarbital.

Fifteen minutes after the injection of

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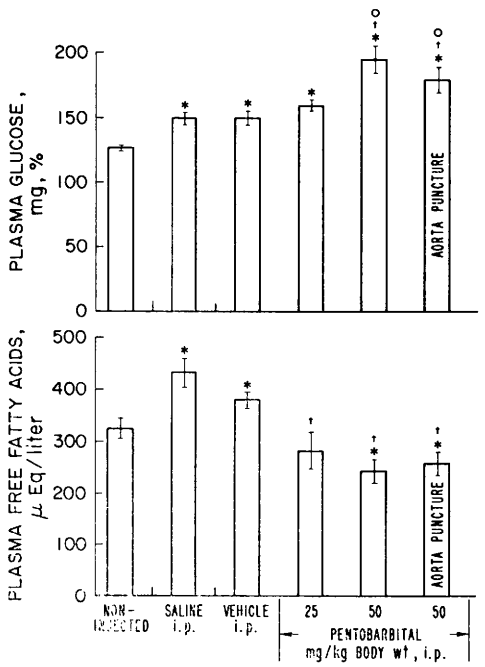

FIG. 1. Plasma glucose (upper) and free fatty acids (lower) in rats 15 min postinjection of saline, Nembutal vehicle, or pentobarbital. Blood was collected by decapitation except for the aortic puncture. (*) $p<.05$, significantly different from noninjected controls. (†) $p<.05$, significantly different from saline and vehicle injected group. (○) $p<.05$, significantly different from 25 mg/kg of body wt; $N = 6 \pm$ SEM.

saline or the Nembutal vehicle, plasma FFA significantly increased to 426 and 383 μEq/liter, respectively, compared to 325 μEq/liter in noninjected controls (Fig. 1). The injection of pentobarbital (25 mg/kg body wt) decreased the plasma FFA to 281 μEq/liter, significantly less than that in saline- or vehicle-treated rats but not significantly different from that of noninjected controls. When the pentobarbital was increased to 50 mg/kg of body wt, plasma FFA decreased to 235 μEq/liter, a value significantly lower than that for noninjected controls. Plasma obtained by aortic puncture had an FFA level of 262 μEq/liter, which was comparable to the level found in decapitated rats that received pentobarbital.

Shown in Fig. 2, is a time course of plasma glucose from decapitated rats following a single injection of 25 or 50 mg/kg of body weight of pentobarbital or the Nembutal vehicle. All three injections significantly elevated plasma glucose compared to noninjected controls for the 5 and 15 min postinjection times with the maximum level occurring at 15 min for all three types of treatment. At 15 min postinjection, the rats which had received 50 mg/kg of body weight of pentobarbital had a significantly higher plasma glucose than did the rats which received the Nembutal vehicle treatment or 25 mg/kg of body weight of pentobarbital.

At 5 min postinjection, plasma FFA were significantly higher in Nembutal vehicle-treated rats or sodium pentobarbital-treated rats than in the noninjected controls (Fig. 3). At 15 min, plasma FFA in the vehicle-treated rats remained significantly higher than in the noninjected controls; however, the 25 and 50 mg/kg of body weight of pentobarbital treatment significantly decreased the plasma FFA compared to vehicle-treated rats. The values were significantly less than in noninjected controls for the 50 mg/kg pentobarbial treatment and remained significantly less through 1 hr postinjection. A significant difference in plasma FFA was found between the 25 and 50 mg/kg of body weight treatment at 30 min.

Discussion. The data show that using pentobarbital for the purpose of anesthesia is contraindicated if the object of the experiment is quantitative analysis of plasma glucose or free fatty acids. As early as 1951, Kimura and DeBoer (4) reported that sodium barbital caused hyperglycemia in rabbits. In 1968, Rodriquez-Torres and Berkovich (5) found that sodium pentobarbital caused hyperglycemia in the African green monkey. We have also found hyperglycemia in the rat as a result of administering sodium pentobarbital. The response is due in part to the injection *per se* as shown by the higher glucose levels found in sham-injected rats as compared to noninjected controls. However, the addition of pentobarbital to the injection media causes a further increase in plasma glucose. Furthermore, the 50 mg/kg of body weight injection of pentobarbital causes a greater increase in plasma glucose than does the 25 mg/kg of body weight dose. It is

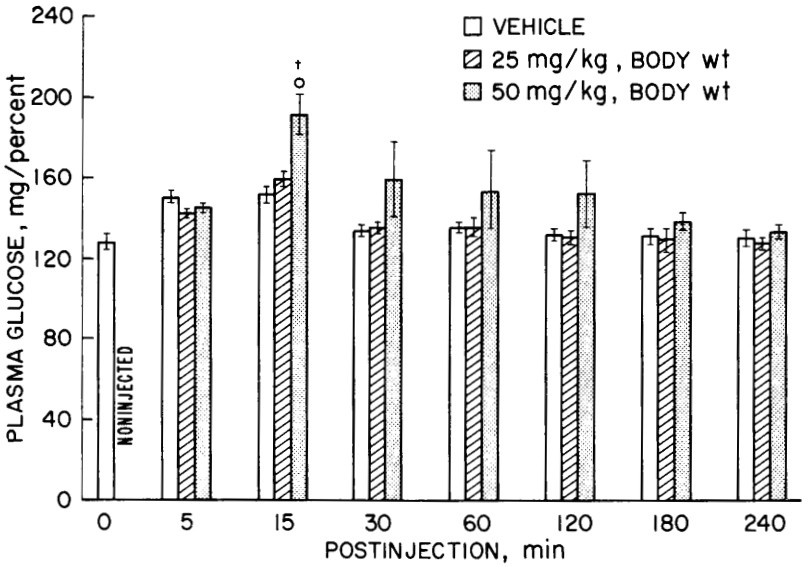

FIG. 2. Plasma glucose in rats at various times after the injection of pentobarbital or vehicle. Each point is the mean of 5 animals for the 25 mg/kg; 7 animals for the 50 mg/kg; and 6 animals for the vehicle and noninjected controls. (+) $p<.05$, significantly different from vehicle treated; (O) $p<.05$, significantly different from 25 mg/kg of body wt.

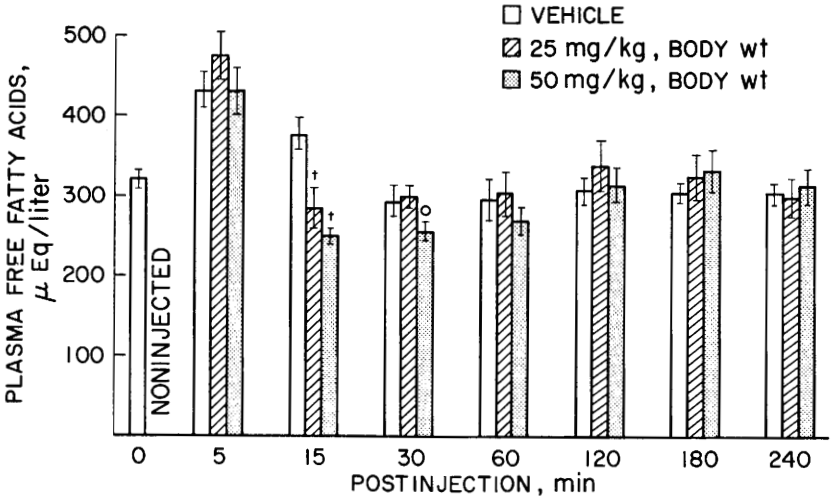

FIG. 3. Plasma free fatty acids in rats at various times after the injection of pentobarbital (+) $p<.05$, significantly different from vehicle treated; (O) $p<.05$, significantly different from 25 mg/kg of body wt. Number of animals as in Fig. 2.

interesting to note that with the higher dose of pentobarbital, the standard errors increase 3–7-fold, which may be due to individual animal sensitivity to the drug and/or the individual variation in the rate at which pentobarbital is metabolized.

Plasma FFA levels were elevated by the injection process as indicated by the saline and vehicle injections. However, when pentobarbital was included in the injection medium, plasma FFA quickly fell to levels below noninjected controls. In the case of the 50 mg/kg of body weight injection, plasma FFA fell below noninjected controls and were also

significantly less than rats receiving 25 mg/kg of pentobarbital. Thus, the level of plasma FFA in the pentobarbital-treated rats is dose dependent. A possible mechanism of action for pentobarbital on plasma FFA is the influencing of the activity of 3′,5′-cyclic AMP (6), which is partially responsible for the control of lipase activity. Further experiments are necessary to establish this mechanism.

Although the effects of pentobarbital disappear within 1 hr, the maximum effect of the drug occurs at 15 min postinjection, a time at which the animal is anesthetized and easiest for the investigator to handle. Thus, carefully selected conditions must prevail for comparison with controls.

Summary. Hyperglycemia and hypolipemia were observed in rats after the injection of sodium pentobarbital (Nembutal). The observed changes were independent of whether the blood was collected by decapitation or by needle puncture of the aorta. The hyperglycemic response was caused by two factors, the stress of the injection *per se* and the pharmacological action of the drug. Hyperlipemia was observed at 5 min postinjection; however, pentobarbital decreased plasma free fatty acids by 15 min postinjection. Both the hyperglycemia and hypolipemia responses were dose dependent.

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