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Hormone and Glucose Responses to Serial Cardiac Puncture in Rats (38465)

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Recent studies have demonstrated that stress from the method used in collecting blood may engender abnormal levels of ACTH (1), corticosterone (2), epinephrine (3), glucose (4), growth hormone (GH) (5) and prolactin (6). Some collection techniques (7), such as cannulation of the jugular veins and running the tubing to an overhead counterbalance system, seem to reduce stress from blood collection, but the system is elaborate and not easy to maintain. Sampling from the tail vein of rats (8) often requires that a heat lamp be used to dilate the vessels, which takes time (thus stressing the animal) and yields only small quantities of blood. Decapitation provides large amounts without stress, but the animal is lost. Cardiac puncture provides enough blood for several assays, and is quick and easy once the animal is anesthetized (9), but problems may result from the type of anesthesia and the duration of exposure. Pentabarbital and the 7- to 15-min wait for it to produce anesthesia elevate GH (10), and a 2-min exposure to ether and then waiting several minutes for sampling depress GH levels (11) while ether exposure increases prolactin levels (12).

In the present study, exposure to high-vapor-saturated levels of ether (causing a fast knockdown) followed by cardiac puncture was investigated as a method of reducing the stress of blood collection from rats. Blood was obtained several times over 24 hr from the same animal and analyzed for several stress-responsive hormones.

Materials and Methods. Experiment 1. Male Sprague–Dawley rats (180–220 g) were housed two/cage under a Light: Dark (L: D) ratio of 14:10 with lights on at 0500 hr. Food was removed at 0700 hr on day 1, returned at 1900 hr, and removed again at 0700 on day 2. All rats were given water *ad libitum* throughout the experiment. Care was taken to minimize various non-specific stressful stimuli.

The rats were divided into two groups: Group C-1, controls, eight animals decapitated at each sampling time and Group E-1, ether knockdown and immediate blood sampling by cardiac puncture (eight animals). In all groups, blood was sampled according to the following schedule: day 1 at 0800, 1400, and 1800 hr; and day 2 at 0800 hr.

At the time of sacrifice or serial cardiac punctures, the animals were taken into an adjacent room. The controls were decapitated within 15 sec of removal from the cages, and systemic blood was collected in heparinized tubes. For the ether-anesthetized rats, several anesthesia jars were used so that the saturation levels of ether would remain high and most rats were unconscious within 30–40 sec of entering the jar. In Group E, almost all blood sampling was complete in less than 60 sec after the animals entered the jar, and in no case in more than 80 sec. An aliquot of 0.75 ml of blood was collected from the heart-punctured animals by means of a heparinized syringe. After blood collection, the animals were injected subcutaneously with 0.75 ml of saline to maintain fluid balance. All blood was iced and centrifuged, and the plasma stored at $-20°$ until assayed.

Insulin was determined by the double antibody method of Manns and Boda (13). Growth hormone and prolactin were determined with materials and methods supplied by the Hormone Distribution Officer, National Institute of Arthritis, Metabolism, and Digestive Diseases. The standard used in the GH assay (NIAMDD Rat-GH-RP-1) had a biological potency of 0.6 IU/mg. Samples were assayed undiluted and, at dilutions of 20 and 50 %; the means were calculated and averaged from the different dilutions used. When known external standards were assayed at the above dilutions the diluted samples paralleled the standard curve and varied about three percent from the expected. The stand-

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TABLE I. PLASMA INSULIN, GH AND PROLACTIN VALUES COLLECTED OVER A 24 HR PERIOD FROM DECAPITATED CONTROL RATS (GR C-1 AND C-2), ETHER ANESTHETIZED HEART-PUNCTURED RATS (GR E-1 AND E-2) AND TWICE ETHER ANESTHETIZED HEART-PUNCTURED RATS (GR 2-EE) ± SE.

Time	0800 hr	1400 hr	1800 hr	0800 hr
Group	Insulin ng/ml			
C-1	2.17 ± 0.07	1.92 ± 0.1	2.08 ± 0.08	2.06 ± 0.14
E-1	2.18 ± 0.10	2.24 ± 0.16	2.13 ± 0.08	2.51 ± 0.05
	N.S.[a]	N.S.	N.S.	$P < 0.025$
C-2	2.68 ± 0.18	2.11 ± 0.22	1.37 ± 0.10	1.20 ± 0.13
E-2	2.74 ± 0.27	1.85 ± 0.23	1.74 ± 0.17	1.22 ± 0.15
	N.S.	N.S.	N.S.	N.S.
	Growth hormone[b] ng/ml			
C-1	163.5 ± 31.2	99.4 ± 34.6	85.2 ± 21.0	133.7 ± 36.4
E-1	229.2 ± 52.5	147.7 ± 33.9	112.6 ± 19.0	204.6 ± 31.0
	N.S.	N.S.	N.S.	N.S.
C-2	170.5 ± 31.0	66.0 ± 21.7	109.2 ± 28.7	184.9 ± 41.0
E-2	186.1 ± 26.9	65.2 ± 27.5	93.3 ± 16.2	119.2 ± 27.8
	N.S.	N.S.	N.S.	N.S.
E-E-2	51.2 ± 7.9	55.5 ± 15.3	29.6 ± 8.5	32.8 ± 4.44
	$P < 0.01$	N.S.	$P < 0.01$	$P < 0.01$
	Prolactin ng/ml			
C-1	15.0 ± 2.6	32.8 ± 3.5	14.1 ± 1.8	21.6 ± 3.3
E-1	68.1 ± 20.0	86.6 ± 7.5	78.0 ± 17.0	79.9 ± 16.2
	$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.01$

[a] N.S. = nonsignificant.
[b] Statistical comparison was made between treatment groups and controls only.

ard used in the prolactin assay (NIAMDD Rat Prolactin-RP-1) had a biological potency of approximately 11 IU/mg. Glucose was determined enzymatically by glucose oxidase using materials and methods supplied by Worthington Biochemical Corp.

Experiment 2. The growth hormone values obtained in Experiment 1, showed considerable variation within the groups. Therefore we decided to repeat the study using a larger number of animals and adding one more treatment group. The three groups were: Group C-2, 16 animals decapitated at each sampling time; Group E-2, ether knockdown and immediate blood sampling by cardiac puncture (16 animals); and Group E-E-2, ether knockdown, 15-min recovery followed by a second ether knockdown with blood sampling by cardiac puncture (16 animals). Growth hormone and insulin were assayed in these groups. The data were analyzed statistically by analysis of variance and the Student's t test.

Results. Insulin. Insulin values did not differ significantly between Groups C-1 and E-1 except at 0800 hr on day 2 (Table I). In Experiment 2 insulin values did not differ significantly between Groups C-2 and E-2 at any of the four bleeding times.

Growth hormone. Growth hormone values of Group E-1 and Group E-2 were not significantly different from those of the control groups (Table I). The GH values of Group E-E-2 were significantly lower than the control values at 0800 and 1800 hr on day 1 and 0800 hr on day 2 but not at 1400 hr on day 1.

Prolactin. Prolactin values were significantly ($P < 0.01$) higher in Group E than in Group C at all four bleeding times (Table I). This elevation was very rapid, as seen in the first sampling on day 1 at 0800 hr: 68.1 ±

TABLE II. PLASMA GLUCOSE VALUES EXPRESSED AS MG/100 ML ± SE OVER 24 HR FROM DECAPITATED CONTROL RATS (GR C-1) AND ETHER ANESTHETIZED HEART-PUNCTURED RATS (GR E-1).[a]

Time	0800 hr	1400 hr	1800 hr	0800 hr
Group				
C-1	130.2 ± 5.3[b]	130.3 ± 2.0[b]	117.8 ± 2.0[b]	130.2 ± 5.3[b]
E-1	126.4 ± 1.8[b]	145.1 ± 2.9[c]	148.7 ± 3.5[c]	146.0 ± 1.8[c]

[a] Mean concentration at each sampling time of control and treatment groups with different superscripts are significantly different ($P < .01$).

20 ng/ml in Group E, and 15.0 ± 2.6 ng/ml in Group C.

Glucose. Glucose values did not differ significantly between Groups C and E at 0800 hr on day 1 (Table II), though glucose in Group E was significantly higher at 1400 hr and 1800 hr on day 1, and again at 0800 hr on day 2.

Discussion. Several hormones that are affected by stress, including ACTH (1), corticosterone (2), and epinephrine (3), have been reported to affect insulin secretion by causing changes in blood glucose levels or by acting directly on the pancreas (14). The data here show that insulin levels were not affected by the methods used to collect blood. The reason, we assume, was that either the method was fast enough and untraumatic enough to avoid stress responses or, there was not sufficient time for stress to be manifested by changes in insulin level.

Schalch and Reichlin (5) reported that cardiac puncture following 2–3 min of ether anesthesia depresses GH in male rats. In this study, apparently basal GH levels were obtained from animals exposed on average to ether vapor for only 30–40 sec. Thus, length of exposure to ether may be an important factor in determining whether GH values are depressed. Garcia and Geschwind (15) reported that the biologic half-life of rat GH is approximately 7.5 min. Thus, at least one sample can be taken without obtaining depressed GH values if ether anesthesia is done quickly, for physiological removal of circulating GH would take a few minutes even if the pituitary release of GH had stopped. The fact that low GH samples were obtained from Group E-E-2 at some of the sampling times suggests that the very short exposure to ether in some cases caused a depression of growth hormone levels. However, this effect must have been very transient and not traumatic enough to influence subsequent blood levels of growth hormone. Clearly, several blood samples can be obtained with normal plasma GH levels from the same animal in a single day when anesthesia is fast and bleeding times are sufficiently spaced.

Wakabayashi *et al.* (12) recently demonstrated that ether stress for 1 min and sampling 2.5 min later caused significant increases in prolactin levels. Our results also show that prolactin release in response to ether stress is very rapid (levels were elevated significantly within 60–80 sec of first exposure to ether).

While both a prolactin-releasing factor (16–18) and a prolactin-inhibiting factor (19) appear to exist, the time course involved in the ether-stimulated release of prolactin in this study is so rapid that it is impossible to suggest which, if either, of the two mechanisms may mediate the effect of stress. Howard and Martin (20) recently showed that sodium pentabarbital caused a direct (*in vitro*) release of GH from the pituitary. The possibility exists that ether may also act directly on the pituitary to cause prolactin release.

Glucose values were elevated at the second, third, and fourth bleedings, whereas insulin was not changed. It is possible that the animals 'learned' they were going to be stressed, so that removal from the cage the second and subsequent times led to a massive sympathetic discharge causing an elevation of blood glucose (3, 4). Montague and Cook (21) have reported that epinephrine can inhibit glucose-induced insulin secretion. Any change in blood glucose level would probably

be of short duration. Insulin release, therefore, may not have been stimulated or, even if insulin release was stimulated blood sampling was completed before detectable changes in insulin could occur.

Summary. Serial, apparently basal radioimmunoassayable plasma insulin and growth-hormone values were obtained when fast-knockdown ether anesthesia was used. Radioimmunoassayable prolactin was elevated at the second, third, and fourth bleedings but cause no apparent increase in circulating insulin levels. The elevation in plasma glucose may also have been a transient effect of sympathetic stimulation which simultaneously inhibited insulin release.

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