NOTE

Effect of Acute Exposure to Cold on the Levels of Corticosterone and Pituitary Hormones in Plasma Collected from Free Conscious Cannulated Rats

AKIRA TAKEUCHI, AKIO KAJIHARA AND MITSUO SUZUKI

First Department of Medicine, School of Medicine, and Department of Physiology, Institute of Endocrinology, Gunma University, Maebashi 371, Japan

Synopsis

To investigate the initial response of the pituitary and adrenal cortex functionings of rats acutely exposed to cold, we developed a technique for cannulation which allowed sequential blood collection from conscious and freed rats. Two cannulae were inserted, one into the carotid artery used for collection and the other into the femoral vein for blood transfusion to keep the blood volume constant. Significant increases in plasma corticosterone and thyrotropin were evoked within 15 min after exposure. While plasma growth hormone (GH) slightly decreased, and the prolactin level tended to fall by 1 hr after cold exposure but the difference was not significant as compared with control values.

It has been established that cold exposure acutely activates the pituitary-adrenal and -thyroid axes (Brown-Grant et al., 1954; Schöbaum, 1960; Itoh et al., 1966; Suzuki et al., 1967; Suzuki, 1971; Jobin et al., 1975).

A circadian periodicity in circulating corticosterone, ACTH and thyrotropin levels is also reported (Guillemin et al., 1959; Cheifetz et al., 1968; Hiroshige et al., 1969; Fukuda et al., 1975). Moreover, recent evidence has indicated that rat pituitary hormones such as LH (Blake, 1975) and growth hormone (GH) (Martin et al., 1974; Tannenbaum et al., 1976) are secreted in pulsatile fashion. These facts explain the wide variability in plasma pituitary hormone levels and indicate the importance of longitudinal studies for the assessment of effects of a given experimental procedure. In a study of GH secretion, Schalch and Reichlin (1966) demonstrated the importance of distinguishing the difference between the effect of blood sampling procedures per se such as handling, puncture and type of anesthesia, and the influence of experimental stimuli being examined. In the majority of these studies, blood was collected by decapitation or even by heart puncture under light anesthesia when small experimental animals such as rats were employed. To avoid these side effects, we developed a technique for cannulation allowing sequential collection of blood samples from rats. In the present paper, we describe the changes in adrenocortical and pituitary hormones in blood collected with the use of this cannulation method from rats acutely exposed to cold.

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**Materials and Methods**

Male Holzman rats, weighing 300 to 350 g were used. They were housed in a temperature controlled (24±1°C) and artificial illumination (light on from 7 a.m. to 7 p.m.) room. They were maintained on pellets of Oriental Yeast Co. and water ad libitum. These animals were handled daily for 60 days before experiment. The animals were divided into three groups, the first group being used for collecting blood for transfusion. The animals were treated with dexamethasone and pentobarbital sodium to stabilize the pituitary and adrenal axis and the trunk blood was collected by decapitation (Kubota and Suzuki, 1974). The second and third groups were used for the experiment on cold exposure. The animals were anesthetized with pentobarbital sodium (20 mg/kg) ip injected before operation. All animals were operated on to insert two cannulae (polyethylene tube, Intramedic-7410, I.D. .023" × O.D. .038") into the carotid artery and femoral vein. The cannula inserted into the right carotid artery was passed through a subcutaneous tunnel to few centimeters below the nape of the neck at the midline where the tubing was fixed to the skin by ligation as shown in Fig. 1. Another cannula was inserted into the right femoral vein and led through a subcutaneous tunnel to the middle back at the midline at where the tubing was fixed (Fig. 1). The cannulae were filled with physiological saline containing 50 U of heparin and tightly covered with a sealed polyethylene tubing Intramedic 7420 (I.D. .034" × O.D. .065"). The cannula inserted into the carotid artery was used for collecting blood and the other for transfusion. The free ends of the two cannulae on the back were loosely fixed at the middle to prevent damaged to the cannulae by biting (Fig. 1).

After the operation, the rats were placed in individual cages for 24 hr. Before cold exposure, the cannulated rats received transfusion of their own blood (autotransfusion) four times in 2 hr, it having been found in a preliminary experiment that the repeated autotransfusion lowered plasma corticosterone to a resting level. Keeping blood pooled at the room temperature and a slow transfusion rate also reduced variability in the adrenal response. Since most of the cannulated rats were quite docile when blood was being collected and transfused, it was quite easy to carry out these procedures without direct handling.

The second group as a control was employed and the third group was exposed to cold. A blood sample (1 ml) was collected from each of the groups and the same volume of blood previously collected was immediately transfused. Rats of the third group were removed to a cold room (2±1°C). The experiments were performed in the same time period (4 p.m. to 10 p.m.). After collecting blood samples, the thyroid glands were quickly removed, weighed and fixed in Bouin's solution for histological study. The thyroid sections were stained with the PAS technique. Histological examination revealed that slight ischemic changes were observed in a maximum 10% of the square in the lobe on the left side, into which the cannula had been inserted, this probably being due to the treatment. However, there was no significant change in hemithyroid weight per body weight.

Corticosterone concentration in plasma was determined after the method of Moncloa et al., (1959) with the use of a Farrand MK I Spectrophotometer. Plasma thyrotropin, GH and prolactin were measured with the aid of radioimmunocassay kits for rat pituitary hormones given by NIAMDD.

Statistical analysis was performed using Student's t test.

**Results and Discussion**

There was a significant increase in the plasma corticosterone level 15 min after the animals were first exposed to cold (Fig. 2). However, in the control experiment, the level did not change significantly during the course of the experiment and the 0 time value (4 p.m.) was nearly as low as the basal or resting level reported by Guillemin et al., (1959). The peak of the average value in cold was observed at 30 min after exposure. There is an agreement between the present finding and the previous observations (Suzuki, 1971; Jobin et al., 1975).

So far, several methods for repeated
blood sampling from small animals have been attempted. In their proper aims, some degree of success could be achieved by repeated cardiac puncture or the use of multiple venipunctures, but both of these techniques have well-known limitations (Jacob and Adriaenssens, 1970). Recently, a few methods for collecting blood samples in unanaesthetized condition have been reported (Martin et al., 1974; Robert, 1975). The latter author developed a special micro-T-shaped cannula inserted into a carotid artery by which both injection and blood withdrawal were made possible but this procedure needed skillful handling. Thus it had to be determined whether the handling resulted in any stressful effect on the animals. On the other hand, Martin et al., (1974) reported a method for permanent indwelling of cannula into the jugular vein. This needed special equipment but made it possible to collect blood samples without direct handling. Our present method did not need any special installation but was found fairly satisfactory for blood collection at the low corticosterone level. To prevent a drop in the hematocrit reading and to permit removal of multiple samples without hemodynamic disturbance, Martin et al., (1974) resuspended red blood cells in saline and returned them to the animals after separating plasma by centrifugation. Obviously, this should cause hypoproteinemia which might induce an unknown disturbance on some, if not all, of the somatic functions. We transfused the same volume of blood as that removed each time, and this kept the hematocrit values constant before and after experiment. In this experiment the probable effect of transfusion on changes in plasma concentrations of corticosterone, thyrotropin, GH and prolactin was felt to be slight, because the volume of blood transfused was small, compared with the total blood volume which is about 4.6 cc/100g body weight (Berlin et al., 1949) and also the disappearance rates for these hormones were quite high (Fortier, 1958; Bakke and Lawrence, 1962; Frohman et al., 1970).
It is widely accepted that stressful stimuli inhibit thyrotropin secretion (Vigneri et al., 1975) and also to exert a marked inhibitory influence on the release of GH (Schalch and Reichlin, 1966). Recent observations have shown that the secretion of prolactin was highly labile and was altered by stress (Neill, 1970).

There was a marked rise in thyrotropin at 15 min after exposure to cold, although no significant change was observed during the course of the control experiment (Fig. 3). The response in the thyrotropin secretion to cold exposure seemed to show a biphasic pattern on the average, in which first and second peaks were observed at 30 min and 2 hr after exposure, respectively. The last one appeared in an earlier phase than that found by Hefco et al. (1975). Average thyrotropin level at 2 hr after exposure was greater than that at 30 min. After 2 hr, plasma thyrotropin level gradually decreased, but was still higher after 5 hr than that of the initial level. The peak of thyrotropin level in individual rats was distributed over from 30 min to 3 hr after exposure to cold. Peak values ranged from 4.5- to 9.0-fold of the initial levels.

The present results showed that cold exposure for 2 hr produced a significant reduction in serum GH, although some controversial results had been reported by Schalch and Reichlin (1968) and Collu et al., (1974) who found a decrease, and by Mueller et al., (1974) who observed no significant change in plasma GH, compared with the control values (Fig. 4). However recent findings on pulsatile secretion of GH seemed to indicate a necessity to make more precise longitudinal estimations.

We were unable to estimate plasma prolactin in some instances, due to an insufficient volume of plasma sample. Therefore, changes in plasma prolactin level after exposure to cold were shown in three rats compared with three normal rats respectively. Plasma prolactin decreased gradually but not significantly after exposure to cold (Fig. 5). There are also controversial reports on the effect of cold exposure to prolactin secretion, in which Mueller et al., (1974) found a significant decrease, while Jobin et al., (1975) observed a marked and transient rise in plasma prolactin. Possibly unknown factors other than blood collecting techniques may account for
Fig. 4. Effect of acute exposure to cold on plasma GH level of rats. Legends are the same as in Fig. 2. a, Significantly different from the 0 time, \( p < 0.05 \).

Fig. 5. Effect of acute exposure to cold on plasma prolactin level of rats. Legends are the same as in Fig. 2.
the difference observed. However, we cannot yet explain these and present findings but further precise studies will be required, as in the case of GH secretion. Recently, it was shown that a single iv injection of synthetic thyrotropin-releasing hormone (TRH) could stimulate a rapid rise in serum prolactin in rats (Mueller et al., 1973), while in another study, a physiological stimulator such as suckling, which is specific for prolactin release elevated serum prolactin unaccompanied by an effect on the circulating thyrotropin level in women (Gautvik et al., 1974). The results of these two physiological studies disclosed that TRH secretion alone is not necessarily responsible for the physiological release of prolactin.

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References