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Effect of Chlorpromazine on Rat Fat Metabolism. (32439)

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Since the advent of phenothiazine therapy chronic administration of chlorpromazine (CPZ) in man has been noted to be associated with an increase in body weight(1,2). Two different mechanisms have been suggested as causes of this phenomenon. Hülsmann *et al* (3) demonstrated that CPZ *in vitro* inhibited the ability of lipases from lung and adipose homogenates of the rat to produce glycerol from fat, and suggested that this inhibition might be responsible for the marked increase in weight seen in patients receiving this drug. Recently studies from two laboratories demonstrated that high doses of CPZ (5×10^{-3} to 1×10^{-3} M) *in vitro* reduce the norepinephrine (NE) induced free fatty acid (FFA) release from rat epididymal fat pads (4-6). This inhibition of FFA release might also be interpreted as contributing to adiposity, but such a mechanism has not been shown to be associated with the *in vivo* administration of CPZ.

In addition to weight gain CPZ administration is known to produce hypothermia in various experimental animals(7-10). The degree of hypothermia increases as the ambient temperature falls(9,11,12), but the temperature of the animals will remain normal at approximately 31°C environmental temperature(13). Hyperglycemia(11), decreased oxygen consumption(8), decreased glucose consumption(11), and inhibition of protein synthesis *in vivo*(12) seen after acute CPZ administration are secondary to this hypothermia and return to normal with proper maintenance of the animals' temperatures.

The present paper presents studies on the effect of *in vivo* administration of CPZ on glucose uptake and glycerol release *in vitro* by rat epididymal adipose tissue from donors with and without hypothermia. Data are provided which demonstrate that the hypothermia induced by the *in vivo* administration of CPZ in rats is associated with increases in fat pad weight and glucose uptake, and decreases in glycerol release.

Methods. Male white Charles River rats weighing between 200-300 g were fed rat chow pellets (Purina) and tap water *ad libitum*. Animals were housed either in pairs in wire cages (8″ × 13½″ × 6″) or individually in plastic cages (5″ × 6″ × 10½″). Each animal was weighed and handled at least 3 days prior to each experiment. All animals were housed in our facilities for at least 6 days prior to the studies on a light-darkness alternated 12 hr schedule. Each study was initiated at 9:30 to 11 a.m. when, after weighing, the animals were injected i.p. with either 20 mg of CPZ per kg body wt (Thorazine®, Smith, Kline and French*) in commercial vehicle or the vehicle without CPZ, and food was removed for those animals scheduled for fasting. CPZ and placebo injections were repeated daily at 9:30 to 11

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a.m. except for the day of sacrifice. For the 48-hour studies involving temperature recording, rats were maintained either at room temperature or within a lighted, air-warmed chicken brooder (Farm Master®, Sears, Roebuck Co.) within which the temperature was maintained at 31°-32°C. Temperatures were recorded prior to injection and at 6 consecutive hourly intervals after injection by the insertion of a small animal rectal thermistor probe to 4 cm and reading the temperature with a Tele-thermometer® (Yellow Springs Instrument Co.). Animals were sacrificed by a blow on the head and severing the neck to permit exsanguination. Epididymal fat pads were removed quickly with excision as close to the epididymus as possible.

Krebs-Ringer bicarbonate buffer (pH 7.4) was prepared according to Cohen(14) containing half the indicated concentration of calcium as recommended by Rodbell(15) and 6% bovine serum albumin (Fraction V—Armour Pharmaceuticals) which had been dialyzed against 50 volumes of the buffer. The weighed fat pads were incubated in an Eberbach water-bath shaker in 20 ml plastic vials at 37°C. Each vial contained 3.0 ml of 6% serum albumin-bicarbonate buffer freshly gassed with 95% O_2 and 5% CO_2 just prior to use. Glucose, obtained from the National Bureau of Standards, was added to make a final concentration of 1 mg/ml. NE was added to make a final concentration of 1 μg/ml. Glycerol was determined by the method of Korn(16), and glucose by the method of Fales *et al*(17).

Results. Fat pads from fed rats, paired in cages and sacrificed 24 hours after daily i.p. injections of CPZ (20 mg/kg body wt), showed significantly greater glucose uptake at 48 hours ($p<0.05$), 72 hr ($p<0.001$), and 96 hours ($p<0.05$) than controls (Fig. 1). Because animals treated with CPZ ate less than controls and lost weight, the effect of fasting was studied.

Fasting did not alter significantly the glucose uptake of fat from control animals at any time period, but did alter the response of the fat from fasted CPZ injected animals in that the only significant increase (CPZ fasted *vs* placebo fasted) in glucose uptake occurred

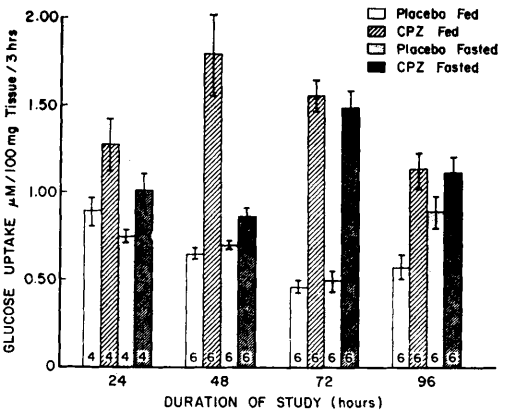

FIG. 1. *In vitro* glucose uptake of adipose tissue from fed or fasted animals injected with either placebo or CPZ for various time intervals. Standard errors of the means are indicated by vertical lines at top of bars. The figure in each column indicates number of animals used in each experiment.

at the 72 hr period ($p<0.01$). It is evident from these data that the increased glucose uptake of fat from CPZ treated rats is not a consequence of decreased food intake. It was also an initial observation that the fat pads from animals receiving CPZ tended to be larger than those from animals receiving placebo.

To determine if the decreased loss of adipose tissue fat in the CPZ treated animals was due to inhibition of NE induced lipolysis, one of each of the pair of fat pads from all rats studied was incubated with NE. In no instance was there any significant difference between the degree of NE induced glycerol release of the placebo treated and CPZ treated animals (range of increment in glycerol release in response to NE for placebo, 0.85-1.20 and for CPZ, 0.77-1.26 μM/100 mg tissue/3 hr).

Because CPZ administration may cause hypothermia in rats maintained at room temperatures, the relationship of changes in body temperature to the fat pad metabolism was studied. Twenty-four animals were isolated and maintained either at 22°C or 31°C (the latter temperature prevents hypothermia following CPZ administration). All animals received 2 injections of either placebo or CPZ 24 hours apart and were sacrificed 24 hours after the last injection.

Three hours after injection animals receiving CPZ and maintained at 22°C had a

TABLE I. 48-Hour Effects of Ambient Temperature on CPZ and Placebo Injected Isolated Animals (Mean ± S.E.M.).

Treatment	No. rats	Initial animal wt (g)	Sacrifice animal wt (g)	Δ Temp 1st day (C°)	Δ Temp 2nd day (C°)	Fat pad wt (mg)	μM/100 mg tissue/3 hr Glucose uptake	μM/100 mg tissue/3 hr Glycerol release
CPZ (22°C)	6	250 ± 4	244 ± 6	−3.8 ± .7	−1.9 ± .7	798 ± 51	1.45 ± .06	.02 ± .01
CPZ (31°C)	6	246 ± 3	248 ± 3	− .8 ± .3	− .6 ± .2	573 ± 30	.95 ± .11	.17 ± .04
Placebo (22°C)	6	247 ± 4	267 ± 6	− .6 ± .2	− .4 ± .2	594 ± 64	.92 ± .15	.21 ± .03
Placebo (31°C)	6	244 ± 3	264 ± 8	− .3 ± .2	− .2 ± .1	581 ± 75	.65 ± .12	.28 ± .05

fall in body temperature of 3.8° and 1.9°C in the first and second 24 hour periods respectively (Table I). All other animals, including those injected with CPZ but maintained at 31°C, had no significant drop in body temperature. The pads of these animals maintained at 22°C and receiving CPZ were significantly larger than those of the other groups of animals ($p<.01$). In addition glucose uptake of adipose tissue taken from animals with CPZ induced hypothermia was significantly greater than that from all the other groups ($p<.01$). Finally the glycerol release by the fat pads from the CPZ treated group maintained at 22°C was significantly inhibited ($p<.01$). This increased glucose uptake and inhibition of glycerol release together with the increase in fat pad weight are consistent with increased lipogenesis.

Discussion. The fact that CPZ administration does not result in changes in fat pad metabolism in animals protected against a fall in body temperature excludes CPZ as the sole or direct cause of the observed changes. The findings suggest rather that it is the hypothermia resulting from CPZ administration which activates a phenomenon involving adipose tissue characterized by increased glucose uptake and decreased glycerol release.

The precise mechanisms causing these metabolic changes are not known, but physiological changes known to be associated with CPZ induced hypothermia such as increased blood sugar(18), increased glucocorticoid output(19), and decreased peripheral glucose utilization(11) are relevant. It is also likely that increased catecholamine activity is involved, because hyperglycemia does not occur in CPZ treated animals if they are adrenal demedullated or pretreated with reserpine or phentolamine(11). The increased blood sugar levels following acute CPZ administration may result from decreased peripheral utilization, gluconeogenesis or glycogenolysis associated with increased glucocorticoid or catecholamine activity(20).

The increased glucose uptake and decreased glycerol release by fat tissue observed in this study could be explained by an increase in insulin secretion resulting from the prolonged hyperglycemia following CPZ administration. The basis for the hyperglycemia would presumably have disappeared by this time (24 hours after CPZ administration) but increased insulin may remain bound to tissue (21). Then, upon incubation, the increased amount of insulin bound to adipose tissue could cause the observed increased glucose uptake and decreased glycerol release observed in fat from CPZ treated donors. The increased glucose uptake, decreased glycerol release, and increased fat pad weight associated with CPZ induced hypothermia are thus consistent with the known properties of insulin.

Summary. The effect of *in vivo* CPZ administration on the *in vitro* metabolism of the rat epididymal fat pad has been studied. Animals maintained at 22°C for 48 hours sustain a hypothermia following CPZ and their fat pads show increased weight, increased glucose uptake, and decreased glycerol release. When animals are similarly treated and maintained at 31°C, there is no hypothermia and no difference in fat pad weight or metabolism from those of placebo treated animals maintained at 22°C or 31°C. It is concluded that the metabolic changes observed are a consequence of the hypothermia induced by CPZ administration.

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Effects of Cortisol and Diethylstilbestrol on Growth Hormone Release by Rat Pituitary *in vitro*.* (32440)

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The secretion of growth hormone in response to hypoglycemia and the administration of arginine in man has been shown to be greatly influenced by certain non-pituitary hormones. Corticosteroids decrease growth hormone response to these stimuli in man (1,2) while estrogens and the synthetic estrogenic compound diethylstilbestrol facilitate the release of growth hormone(3,4). The mechanism of action of these modifying effects has not been established. Corticosteroids have little effect on the concentration of growth hormone in the pituitary of the normal rat(5). If corticosteroids are administered during the induction of hypoglycemia, the expected drop in growth hormone content is inhibited(5). Estrogens on the other hand decrease the concentration of growth hormone even when compared to pair fed control rats(6).

The modifying effects of estrogens and corticosteroids on growth hormone secretion could be exerted either at the level of the hypothalamus by affecting the secretion of the somatotropin releasing factor or these hormones could affect the secretion of growth hormone by acting directly on the somatotropic cells themselves. The observations of Pecile and Müeller(5) have suggested that cortisol may deplete the hypothalamus of somatotropin releasing factor. In their experiments hypothalamic extracts from cortisol treated animals had a much reduced capacity to cause a depletion of bioassayable pituitary growth hormone in recipient animals when compared to hypothalamic extracts from normal rats. Cortisol-treated recipient rats retained their ability to respond with a depletion of bioassayable growth hormone when injected with hypothalamic extracts from

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