Studies on the Mechanism Controlling Growth Hormone Release Induced by Chlorpromazine in the Anesthetized Rat

KAZUO CHIHARA, YUZURU KATO, SHOZO OHGO AND HIROO IMURA

Third Division, Department of Medicine, Kobe University School of Medicine, Kobe 650

Synopsis

In intact urethane-anesthetized rats, plasma growth hormone (GH) levels were low but increased significantly following intravenous injection of chlorpromazine. Plasma GH levels were significantly elevated in rats with hypothalamic cuts such as complete deafferentation, anterior cut and antero-lateral cut, whereas plasma GH levels in rats with posterior cut or posterolateral cut were not significantly different from those in rats with sham-operation. Intravenous injection of chlorpromazine caused an increase of plasma GH in rats with any type of hypothalamic cut. However, the maximum increments of plasma GH following chlorpromazine were larger in rats with antero-lateral cut and smaller in rats with posterior cut than in rats with sham-operation. These results suggest that extrahypothalamic inhibiting and stimulating neurons influence the regulatory mechanism of rat GH secretion through anterior and posterior routes to the hypothalamus respectively.

The important role of neural regulation has recently been documented in the regulation of growth hormone (GH) secretion (Müller, 1973). Kato et al. (1973) has demonstrated that chlorpromazine, a possible central dopamine antagonist, caused an increase in plasma GH in rats. We have confirmed these findings and extended the studies in order to clarify the mechanism by which chlorpromazine acts to regulate GH secretion (Kato et al., 1974 a, b). Although the exact mechanism remains to be clarified, chlorpromazine possibly acts at the hypothalamus to modulate the release of either GH releasing factor (GHRF) or GH release inhibiting factor (GHRIF).

The present studies were undertaken to examine the extrahypothalamic influences regulating plasma GH response to chlorpromazine in rats.

Materials and Methods

Male rats of the Wistar strain, weighing 180-220 g, were housed in a constant ambient temperature of 22±2°C, air conditioned room under artificial lighting (lights on at 0700, off at 2100 hr), and were maintained on rat biscuits (Oriental Yeast Co., Tokyo) and water ad lib.

Under pentobarbital anesthesia (4 mg/100 g, i. p.), stereotaxic deafferentation of the medial basal hypothalamus was performed with a modification of the Halász-Pupp knife (Halász et al., 1965), 1.5 mm lateral×1.5 mm vertical, fixed in the holder of a stereotaxic instrument. After the skull was held in a stereotaxic instrument with a lowered toothbar at an angle of 10° inferior of the horizontal zero plane of the De Groot atlas (1959), five types of deafferentation were performed (Fig. 1).

In complete deafferentation, anterior cut and anterolateral cut, the knife was lowered through midline at a point 1.5 mm posterior to the bregma with the
Fig. 1. Placements and dimensions of cuts on the ventral surface of the brain are indicated by the heavy lines on the diagrams: 1. Complete deafferentation, 2. Anterior cut, 3. Postero-lateral cut, 4. Antero-lateral cut, 5. Posterior cut.

Tip facing anteriorly. In complete deafferentation, the knife was rotated 90° to the left, the stereotaxic carrier moved posteriorly 2.0 mm, the knife rotated peripherally 180°, the stereotaxic carrier moved anteriorly 2.0 mm, the knife rotated 90° to the left and removed from the brain at the site of entry. In the anterior cut, the knife was rotated 90° to both sides of the midline. In the antero-lateral cut, the knife was rotated 90° to the left, the stereotaxic carrier moved posteriorly 2.0 mm, the knife and the stereotaxic carrier backed to the starting position, the knife rotated 90° to the right and the stereotaxic carrier moved posteriorly 2.0 mm.

In the posterior and postero-lateral cuts, the knife was lowered through the midline at a point 6.5 mm posterior to the bregma with the tip placed posteriorly. In the posterior cut, the knife was rotated 90° to both sides. In the postero-lateral cut, the knife was rotated to the left, the stereotaxic carrier moved anteriorly 2.0 mm, the knife and the carrier backed to the starting position, the knife rotated 90° to the right and the carrier moved anteriorly 2.0 mm.

The sham operation consisted of lowering the knife through the midline to the base of the brain and omitting rotation.

The experiments, using rats with various hypothalamic cuts, were performed 2 to 3 weeks after surgery.

Fig. 2. Localization and histological structure of the hypothalamus 3 weeks after complete deafferentation. Different coronal plane through the medial hypothalamus are shown. The arrows indicate the cut. Abbreviations are: RCA = retrochiasmatic area, TRO = optic tract, FX = fornix, DM = dorsomedial nucleus, VM = ventromedial nucleus, ARC = arcuate nucleus, ME = median eminence, PMD = dorsal premammillary nucleus, PMV = ventral premammillary nucleus, ST = pituitary stalk, VIII = third ventricle.
After overnight fasting, chlorpromazine or saline was injected into the rats' jugular vein under urethane anesthesia (150 mg/100 g, b.w.). Blood samples of 0.6 ml were withdrawn from the contralateral jugular vein, immediately before, 10, 20 and 40 minutes after the injection. Details of sampling procedures were described in our previous report (Kato et al., 1973). Blood samples were immediately centrifuged and plasma was kept at -20°C until assayed.

The precise location of the deafferentation was evaluated histologically after the experiments. Brains were fixed in 10% formalin, embedded in paraffin, cut into serial sections at 10 µ and stained with hematoxylin-eosin. The complete deafferented hypothalamic island included the arcuate nuclei, periventricular nuclei, most of the ventromedial nuclei and the premammillary area (Fig. 2). Only those animals in which deafferentation was verified histologically as properly placed are incorporated in these studies.

Plasma rat growth hormone was measured in duplicate on 0.1 ml aliquots of plasma using specific radioimmunoassay (Kato et al., 1973). Rat growth hormone for radioiodination (NIAMD-Rat GH-I-1), rat growth hormone reference preparation (NIAMD-Rat GH-RP-1) and monkey anti-rat GH serum (NIAMD-Rat GHS-1) were all kindly supplied by the National Institute of Arthritis, Metabolism and Digestive Diseases, NIH, Maryland.

Student's t test was used in statistical analysis.

Results

Effects of complete deafferentation on basal plasma GH levels and chlorpromazine-induced plasma GH responses

Under urethane anesthesia, basal plasma GH levels were low and stable in rats with sham-operation, whereas they were significantly elevated in rats with complete deafferentation (Mean ± SEM : 4.1 ± 0.8 ng/ml vs 24.5 ± 5.4 ng/ml, p < 0.005, Fig. 3).

Saline injection did not cause any significant changes in plasma GH levels, while the injection of chlorpromazine (200 µg/100 g, b.w.) caused a significant increase in plasma GH, compared with basal levels (p < 0.005) and saline control levels (p < 0.005) in rats with complete deafferentation as well as in rats with sham operation. The peak of plasma GH appeared 40 min after the injection of chlorpromazine in both groups of rats. The maximum increments of plasma GH following chlorpromazine (ΔGH) tended to be larger in rats with complete deafferentation, but not significantly different from those in rats with sham-operation.

Effects of various hypothalamic cuts on basal plasma GH levels

Basal plasma GH levels were significantly elevated in rats with anterior cut (p < 0.05) or antero-lateral cut (p < 0.005) as well as complete deafferentation (p < 0.005), whereas plasma GH remained unchanged in rats with sham-operation (Fig. 4). No significant statistical differences in basal plasma GH levels were demonstrated either among rats with anterior cut, antero-lateral cut and complete deafferentation or between rats with posterior and postero-lateral cuts.

Effects of various hypothalamic cuts on plasma GH response to chlorpromazine

Intravenous injection of chlorpromazine (200 µg/100 g, b.w.) caused a significant increase in plasma GH in rats with anterior, antero-lateral, potero-lateral and posterior cuts as well as complete deafferentation compared with their basal levels (p < 0.025, p < 0.005, p < 0.05, p < 0.05 and p < 0.05, respectively). However, the maximum in-

![Fig. 3. Effects of chlorpromazine (200 µg/100 g, b.w., i.v.) on plasma GH in rats with complete deafferentation or sham operations. Mean ± SEM's are shown. The numbers of animals in each group are indicated in parentheses.](image-url)
crements of plasma GH ($\Delta$GH) were significantly suppressed in rats with posterior cut ($p<0.05$) and significantly enhanced in rats with antero-lateral cut ($p<0.05$), compared with those in rats with sham-operation (Fig. 5). $\Delta$GH in rats with anterior cut tended to be insignificantly larger than those of rats with sham-operation. Also, $\Delta$GH in rats with postero-lateral cut tended to be insignificantly smaller than those of rats with sham-operation.

There was no significant difference in $\Delta$GH either between rats with anterior cut and those with antero-lateral cut, or between rats with posterior cut and those with postero-lateral cut.

Discussion

Plasma GH levels were low and stable under urethane anesthesia in intact rats, compared with plasma GH levels after decapitation (Kato et al., 1973). It may be considered that urethane has an inhibiting effect on the tonic regulation of basal GH levels in rats. We observed that basal plasma GH levels were elevated in the rats with complete deafferentation, anterior and antero-lateral cuts. These findings suggest that inhibitory effects of urethane anesthesia are possibly transmitted through a nervous pathway which may reach the medial basal hypothalamus through an anterior route. Mitchell et al. (1973) demonstrated that surgical isolation of the medial basal hypothalamus caused marked elevation in plasma GH levels in rats under ether anesthesia.

We reported previously that chlorpromazine induced GH release in rats and speculated that this effect was explained by a blockade of midbrain dopamine receptors or by alpha-adrenergic blockade in the hypothalamus (Kato et al., 1973). The present studies demonstrated that chlorpromazine caused a rise of plasma GH in rats with complete hypothalamic deafferentation. Further studies in our laboratory demonstrated that plasma GH response to chlorpromazine was abolished in rats with hypothalamic lesions (Kato et al., 1974 a). These findings suggest that chlorpromazine acts at the level of the hypothalamus.

We also demonstrated in the present studies...
studies that plasma GH responses to chlorpromazine were altered by different types of hypothalamic cuts in rats, which suggests the existense of the extrahypothalamic influences on the regulation of rat GH secretion.

In our experiments, the maximum increments of plasma GH following chlorpromazine were significantly lower in rats with posterior cut, and also significantly higher in rats with antero-lateral cut than in rats with sham-operation. No significant difference was observed between plasma GH responses to chlorpromazine in rats with posterior cut and those in rats with posterolateral cut.

These findings may indicate that the extrahypothalamic inhibitory influence on GH secretion reaches the medial basal hypothalamus through antero-lateral pathways, whereas the extrahypothalamic stimulating influence reaches posteriorly the hypothalamus. Martin (1972) and Smith et al. (1971) demonstrated the extrahypothalamic control of GH secretion in the rat and the monkey. The stimulating and inhibitory afferent inputs to the medial basal hypothalamus were also demonstrated by electrophysiological studies (Deifuss et al., 1968; Nauta, 1963).

Since hypothalamic deafferentation causes a decrease of noradrenaline (Weiner, 1972) and serotonin (Popova, 1972) content in the hypothalamic island in rats, the neurons influencing GH release may contain the biogenic amines which are assumed to be involved in the regulation of release of GHRF or GHRIF (Müller, 1973). However, the exact relationship between the biogenic amines and GHRF or GHRIF remains to be investigated.

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References