Effect of Catecholamines on Plasma Growth Hormone in Dogs

KIYOHISA TAKAHASHI, TOSHIO TSUSHIMA† and MINORU IRIE*

Department of Neurochemistry, Tokyo Metropolitan Institute for Neurosciences, Tokyo, †Third Department of Internal Medicine, University of Tokyo, Tokyo and *First Department of Internal Medicine, Toho University, Tokyo

Synopsis

L-dopa, dopamine and norepinephrine (1 mg/kg) caused a marked increase in plasma growth hormone (GH) when given i.v. to trained, unanesthetized, fasting dogs. Reduction of the dose to 100 µg/kg eliminated the responses. On the other hand, the relatively small dose of epinephrine (10 µg/kg) could increase plasma GH level. In this case a reduction of the dose to 1 µg/kg also diminished the stimulatory effect. A significant increase in plasma glucose accompanied the rise in plasma GH with dopamine, norepinephrine and epinephrine but not with 1-dopa. Furthermore, in the case of dopamine and norepinephrine a concomitant increment of plasma cortisol was also found. Alpha adrenergic blockade with phentolamine suppressed the stimulatory effect of epinephrine and 1-dopa on GH release. These results suggest that GH release stimulated by catecholamines is mediated by alpha adrenergic receptor. An attempt to inhibit dopamine beta hydroxylase with fusaric acid did not alter the stimulatory effect of 1-dopa. This result suggests an important role for dopamine in GH secretion mechanisms.

Catecholamines appear to be deeply involved in the secretion mechanisms of pituitary hormones. Recently ample evidence has been accumulating indicating that gonadotropin and prolactin secretion are regulated by catecholamines (MaCann, 1972). In the case of growth hormone (GH) a number of studies also suggesting a profound relationship with catecholamines have been reported.

Recently several investigators (Boyd et al, 1970 and 1971, Saito et al, 1972, Yoshimi et al, 1972) reported the stimulatory effect of 1-dopa on GH secretion in humans. Large doses of epinephrine have been reported to increase plasma GH in monkeys (Knobil and Meyer, 1968; Gagliardino, 1968). In humans the stimulatory effect of epinephrine on GH secretion persisted in the presence of beta adrenergic blockade (Blackard, 1970). Furthermore, it has been postulated that beta adrenergic blockade stimulates and alpha blockade suppresses GH secretion (Imura et al, 1968 and 1971; Blackard, 1968; Werrbach, 1970).

However, although the studies mentioned above suggest that catecholamines play an important role in the secretion mechanisms of GH, the details are still unclear. Little is known about the role of dopamine and norepinephrine. Besides, there are conflicted data on the effect of epinephrine on GH release in different species. In monkeys two reports agreed about the stimulatory effect of epinephrine. On the other hand, negative results were obtained with epinephrine alone in humans (Rabinowitz, 1966; Schalch, 1967), but positive results were obtained with epinephrine in the presence of beta adrenergic blockade. Furthermore, an obvious suppressive effect of epinephrine was demonstrated by Hertelendy et al (1969) in sheep and in rats by Takahashi et al (1971). These results suggest

Received for publication May 14, 1973.
that there are species differences in GH secretion response to epinephrine.

Recently a radioimmunoassay for canine GH has been developed in our laboratory (Tsushima et al., 1971). The purpose of the present study is to investigate the effect of catecholamine on GH secretion in dogs to observe the mode of its action and to compare the results with those obtained in other species previously reported.

Materials and Methods

All experiments were carried out using adult Japanese mongrel dogs, weighing 8-12 kg, following an overnight fast. Animals were utilized for experiments repeatedly with a 10-14 days intervals between experiments. During experiment, animals were unanesthetized and unrestrained except for a long chain connected to a dog collar. After two hours adaptation to the new environment of the experiment room, test materials were injected into the brachial vein or infused through an indwelling catheter placed in the jugular vein. Blood samples were obtained from the brachial vein by venipuncture or from an indwelling catheter every 15-20 min over 90-180 min after the start of experiment. The blood specimens drawn into heparinized syringes were stored at -20°C until assay. Plasma GH was determined by double antibody radioimmunoassay developed in our laboratory (Tsushima et al., 1971). Highly purified canine GH (D887A: 2.0 IU/mg) prepared by Dr. A. E. Wilhelmi was used for iodination and standard. Plasma cortisol was measured by the competitive protein binding method using corticoid binding protein from plasma obtained from pregnant women (Murphy, 1967). Plasma glucose levels were analyzed by the glucose oxidase method (Huggett and Nixon, 1957).

L-dopa, epinephrine and norepinephrine were supplied from Sankyo Drug Company. Dopamine was obtained from Kyowa Hakko Co. and fusaric acid by Merk Banyu Co. Statistical analysis was done by means of t test.

Results

Effect of L-dopa (Fig. 1)

A single injection of l-dopa (1 mg/kg) produced a prompt increase of plasma GH (p < 0.05). Neither plasma glucose nor cor-
Effect of epinephrine (Fig. 2)

After a single injection of 10 µg/kg epinephrine plasma GH level was elevated within 15 min (p < 0.05). Concomitant rise of plasma glucose from 68 ± 5 mg/100 ml at 0 time to 88 ± 8 mg/100 ml at 15 min was also observed. One tenth of the dose (1 µg/kg) did not produce any change in plasma GH, glucose or cortisol. In the report of Herschel et al (1969), epinephrine suppressed GH secretion caused by arginine infusion in sheep. In that report plasma GH seemed to be elevated after the cessation of epinephrine infusion. To exclude the possibility of such a rebound phenomenon, a dose of 0.5 µg/kg/min of epinephrine was infused over one hour through the indwelling catheter over one hour at the rate of 0.5 µg/kg/min.

EFFECT OF EPINEPHRINE INFUSION

Fig. 3. Effect of epinephrine infusion on plasma GH, cortisol and glucose. Epinephrine was infused through the indwelling catheter over one hour at the rate of 0.5 µg/kg/min.

Fig. 2. Effect of epinephrine injection on plasma GH, cortisol and glucose. Epinephrine was injected at 0 time with two different doses, 10 µg/kg and 1 µg/kg, as indicated at the top left of each figure.
Effect of dopamine (Fig. 4)

Dopamine (1 mg/kg) caused an increase in plasma GH within 15 min (p < 0.05) with concomitant elevation of plasma glucose and cortisol. Reduction of the dose to 100 µg/kg resulted in no appreciable change in GH, glucose and cortisol.

Effect of norepinephrine (Fig. 5)

One mg of norepinephrine increased plasma GH significantly (p < 0.05) within 15 min after the administration. A marked increase in glucose and cortisol was also observed. With 100 µg/kg, however, no significant change was obtained, although three out of seven animals showed an elevation of plasma GH.

Effect of alpha adrenergic blockade with phentolamine (Fig. 6)

Phentolamine (1 mg/kg) administered at 60 and 30 min before the injection of catecholamines suppressed the stimulatory effect of both 1-dopa (1 mg/kg) and epinephrine (10 µg/kg).

Effect of fusaric acid on stimulatory effect of 1-dopa (Fig. 7)

In attempt to inhibit dopamine beta hydroxylase with fusaric acid, 20 mg/kg was given twice intravenously 3 and 2 hr before the 1-dopa injection. As shown in Figure 7, fusaric acid did not alter the effect of 1-dopa (1 mg/kg) on plasma GH.

Discussion

The present study demonstrated that large doses of 1-dopa, dopamine, norepinephrine and epinephrine stimulate GH release in dogs. Epinephrine is the most potent stimulator for GH secretion among the four catecholamines, because 10 µg/kg of epinephrine was effective, while 1 mg/kg of the other three drugs was required to produce an equivalent response.
Fig. 5. Effect of norepinephrine injection on plasma GH, cortisol and glucose. Norepinephrine was injected at 0 time with three different doses, 1000, 100 and 10 μg/kg, as indicated at the top left of each figure.

Fig. 6. Effect of alpha adrenergic blockade with phentolamine on stimulatory effect of 1-dopa and epinephrine on GH secretion. Phentolamine (1 mg/kg) was injected twice, 60 min. and 30 min. before 1-dopa (1 mg/kg) or epinephrine (10 μg/kg) injection. Each point represents mean of 3 experiments with 1-dopa and 4 with epinephrine. Solid line represents the case of epinephrine and dotted line 1-dopa.

Fig. 7. Effect of dopamine beta hydroxylase inhibition with fusaric acid on stimulatory effect of 1-dopa. 20 mg/kg of fusaric acid was injected twice, 3 hr and 2 hr before 1-dopa injection (1 mg/kg). No ready explanation about the difference in the effective doses is forthcoming.

GH secretion was accompanied by an elevation of plasma glucose and cortisol in the case of dopamine and norepinephrine, but not in the case of 1-dopa. From this fact it is possible that 1-dopa stimulate GH secretion.
in a different way from exogenous dopamine and norepinephrine. With our experimental designs it is difficult to distinguish between effects at the central nervous system level and as a result of peripheral stress. As several investigators (Weil-Malherbe et al, 1959 and 1961; Bertler et al, 1966) reported that circulating catecholamines were absorbed by the hypothalamus to some extent, although they did not cross the blood-brain barrier in other regions, one possibility is that catecholamines stimulate GH releasing system at the level of hypothalamus. However, to verify this possibility different experimental designs, such as intraventricular or intracerebral administration of test materials, are required, which are in progress in our laboratory.

Hertelendy et al (1969) demonstrated that epinephrine infusion at the rate of 0.5 μg/kg/min suppressed the stimulatory effect of arginine on GH secretion in sheep and that the GH level was elevated immediately after the cessation of the infusion. Contrary to their results, in our case the exact same infusion method of epinephrine produced a definite increase of plasma GH at the beginning of infusion period. Large dose of epinephrine have been demonstrated to increase plasma GH in monkeys (Knobil and Meyer, 1968; Gagliardino and Martin, 1968). On the other hand, in rats it was reported that epinephrine decreased GH level (Takahashi et al, 1971). These facts suggest that there are species differences in the effect of epinephrine on GH secretion.

The fact that alpha adrenergic blockade with phentolamine suppressed the stimulatory effect of epinephrine and 1-dopa indicates that GH secretion stimulated by these catecholamines is mediated by alpha adrenergic receptors. Since Blackard and Heidingsfelder (1968) reported that alpha adrenergic blockade blunted the stimulatory effect of insulin hypoglycemia on plasma GH in humans, several reports have confirmed the suppressive effect of alpha adrenergic blockade in humans and monkeys (Blackard, 1970; Werrbach et al, 1970; Hansen, 1971; Imura et al, 1971; Massara et al, 1972; Kansal et al, 1972). The present study demonstrated the inhibitory effect of alpha adrenergic blockade on GH secretion also in dogs.

In order to determine whether or not 1-dopa has to be converted to epinephrine resulting to stimulate GH secretion, we attempted to inhibit dopamine beta hydroxylase with fusaric acid. Fusaric acid has been reported to be a potent inhibitor of dopamine beta hydroxylase (Hidaka et al, 1969; Nagatsu et al, 1970). Therefore, large doses of fusaric acid are expected to inhibit the conversion of dopamine synthesized from exogenous 1-dopa to norepinephrine. With 40 mg/kg of fusaric acid, which was sufficient to deplete endogenous norepinephrine (Nagatsu et al, 1970), no appreciable change in the stimulatory effect of 1-dopa on GH secretion was observed. This result suggests that 1-dopa given exogenously does not have to be converted to epinephrine to stimulate GH release and that dopamine may play an important role in GH release in the dog.

Acknowledgments

The generosity of Dr. A. E. Wilhelmi, who provided highly purified canine GH, is gratefully acknowledged. The authors also appreciate the technical help of Miss Fumiko Oyamada and Miss Chieko Hayafuji.

References

**Acta Endocr.** (Kobenhavn.) 59, 390.
Hidaka, H., T. Nagatsu, K. Takaya, T. Takauchi, H. Suda, K. Kojiri, M. Matsu-