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Effects of Drugs Infused into a Rat Hypophysial Portal Vessel on Prolactin and Growth Hormone Release (40965)[1]

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Abstract. To determine the site of action of various drugs which affect the release of prolactin (PRL) and growth hormone (GH), these drugs were infused into a hypophysial portal or jugular vein in anesthetized rats. When infused into a hypophysial portal vessel, L-dopa, a precursor of dopamine, significantly inhibited PRL release, whereas the dopamine antagonists, haloperidol and sulpiride, stimulated PRL release. In contrast, GH release was not significantly changed by any of these drugs. Infusion of substance P into a portal vessel caused an increase in both PRL and GH release. However, systemic infusion of haloperidol, sulpiride, and substance P in the same dose infused into a portal vessel failed to affect both PRL and GH levels. These findings suggest that PRL but not GH secretion is inhibited by a dopaminergic mechanism at least in part at the pituitary level, whereas substance P may stimulate both PRL and GH secretion at the pituitary level.

Recent studies revealed that not only biogenic amines but also small peptides in the brain play an important role in the regulation of prolactin (PRL) and growth hormone (GH) secretion, possibly acting at either the hypothalamus or the pituitary level (1–3). In order to elucidate the site of action of these substances, we investigated the effect of drugs injected directly into a hypophysial portal vessel on plasma PRL and GH levels in the anesthetized rat.

Materials and Methods. Male Wistar strain rats weighing 200 to 250 g (Japan Animal Co., Osaka) were used throughout the experiments. They were maintained in a light (14-hr light, 10-hr dark)- and temperature (25 ± 1°C)-controlled room and fed Oriental laboratory chow (Oriental Yeast Co., Tokyo) and water *ad libitum*.

After an overnight fast, they were anesthetized with urethane (150 mg/100 g body wt ip) and the pituitary stalk was exposed by the parapharyngial approach as described by Porter *et al.* (4). A fine curved glass cannula was inserted into a main portal vessel and the test materials were infused at a rate of 50 ng/2 μl/min for 20 min using an infusion pump (Harvard Apparatus, Mass.). Insertion of the cannula and anterograde flow of test substances during the infusion were monitored under the fields of a binocular microscope (×20) with coaxial lighting. In another experiment, test substances were infused into the jugular vein for 20 min in the same dose as infused into the hypophysial portal vessel.

L-Dopa (Sankyo Pharmaceutical Co., Tokyo), haloperidol (Dainippon Pharmaceutical Co., Osaka), sulpiride (Fujisawa Pharmaceutical Co., Osaka), and synthetic substance P (a gift from Dr. Yanaihara, Shizuoka Pharmaceutical College, Shizuoka) were dissolved in phosphate-buffered saline. Each solution contained a trace dose of lissamine green as a marker dye.

Immediately before the infusion of test materials and at 10- to 20-min intervals thereafter, blood samples of 0.6 ml were withdrawn from the jugular vein using a heparinized tuberculin syringe as described previously (5).

Plasma PRL and GH levels were determined by specific radioimmunoassay (6, 7) with materials supplied from the National Institute of Arthritis, Metabolism and Digestive Diseases. NIAMDD rat prolactin-

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RP-1 and GH-RP-1 were used as reference preparations for each assay. Student's *t* test was used for statistical evaluation.

Results. As shown in Fig. 1., plasma PRL levels were significantly decreased during the infusion of L-dopa into a hypophysial portal vessel (50 ng/min for 20 min) compared with a control group in which phosphate-buffered saline was infused. In contrast, plasma GH levels were not significantly changed by L-dopa infusion into a portal vessel.

Either haloperidol or sulpiride infusion into a portal vessel at a rate of 50 ng/min for 20 min resulted in a significant increase in plasma PRL levels as shown in Figs. 2 and 3. However, plasma GH levels were not significantly changed by the infusion of these drugs compared with control group.

As shown in Fig. 4, the plasma levels of both PRL and GH were significantly raised by substance P infused into a hypophysial portal vessel (50 ng/min for 20 min).

On the other hand, the infusion of haloperidol, sulpiride, or substance P into the jugular vein in a dose and rate identical to those used for the infusion into a portal vessel failed to change plasma PRL and GH levels (Fig. 5).

Discussion. In the present studies, we showed that infusion of L-dopa, a precursor of dopamine, into a hypophysial portal vessel inhibited PRL release from the rat pituitary gland. This is consistent with the finding that intraportal infusion of dopamine decreased PRL secretion in the rat (13), although the mechanisms of an inhibition by L-dopa of PRL release remain unclear. Donoso *et al.* (8, 9) observed that L-dopa decreased the elevated serum PRL levels in rats with lesions in the median eminence and in rats with transplanted pituitaries, whereas pretreatment of rats with a peripheral aromatic L-amino acid decarboxylase inhibitor blunted the inhibition by L-dopa of prolactin release from transplanted pituitaries. This suggests that L-dopa acts after transformation into dopamine. However, it still remains unknown where L-dopa infused into a hypophysial portal vessel is converted to dopamine. Recent studies by Page *et al.* (10) and by Oliver *et al.* (11) suggest that pituitary hormones are transported from the pituitary to the hypothalamus and then back to the pituitary. Therefore, L-dopa infused into a portal vessel might be carried to the hypothalamus by a retrograde back flow, and there converted to dopamine. Alternately, the conversion of L-dopa to dopamine might occur in the anterior pituitary. It was recently shown that L-dopa as

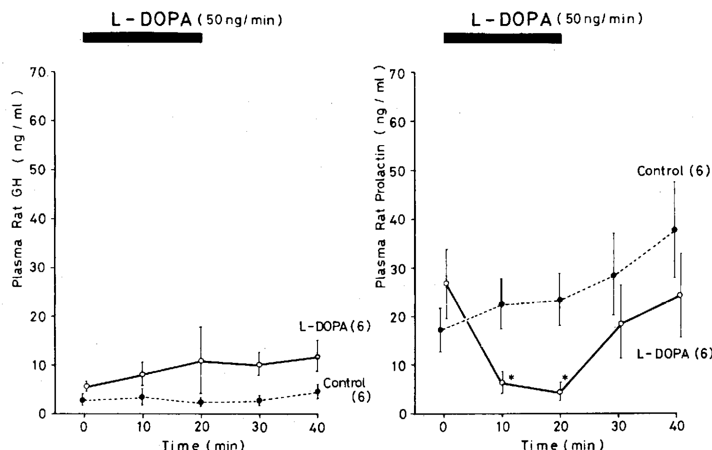

FIG. 1. Plasma GH and PRL levels following the infusion of L-dopa into a hypophysial portal vessel in the rat. L-Dopa was infused for 20 min at a rate of 50 ng/min. Phosphate-buffered saline was infused in a control group. Means ± SE are shown. The number of animals in each test group is indicated in parentheses. Statistical difference (vs control) is shown by an asterisk: $*P < 0.05$.

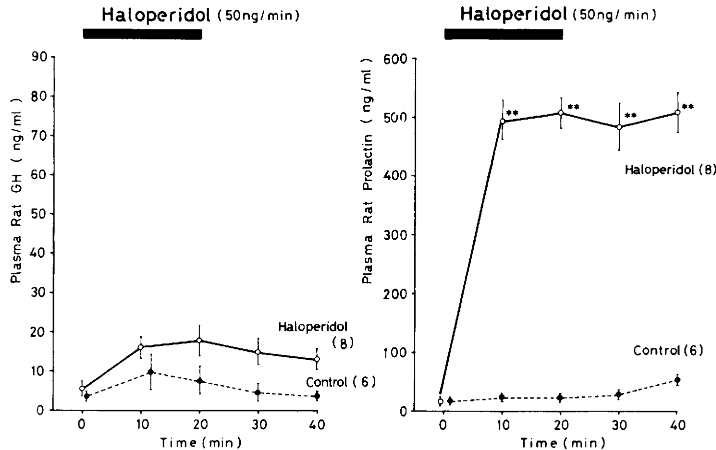

FIG. 2. Plasma GH and PRL levels following the infusion of haloperidol at a rate of 50 ng/min for 20 min into a hypophysial portal vessel in the rat. Statistical difference (vs control) is shown by asterisks: $^{**}P < 0.01$.

well as dopamine was effective in suppressing PRL release from human pituitary adenoma cultured *in vitro* (12).

Ben-Jonathan *et al.* (17) and Gibbs *et al.* (18) have demonstrated that in the rat, hypophysial portal blood contains a concentration of dopamine which is sufficient for inhibition of PRL secretion. In this study, we observed that infusion of the dopamine antagonists, haloperidol and sulpiride, into a hypophysial portal vessel increased PRL secretion from the pituitary. Since these antidopaminergic agents infused into a jugular vein at rates identical to those infused into a portal vessel failed to affect plasma PRL levels, they seem to exert their effects directly on the pituitary gland. In agreement with our finding, Macleod and his associates (14, 15) have demonstrated in their *in vitro* experiments that dopamine inhibited the release of PRL and haloperidol or sulpiride attenuated the inhibitory action of dopamine on PRL secretion. Calabro *et al.* (16) demonstrated that [^{3}H]dopamine bound specifically to the membrane fraction of bovine anterior pitu-

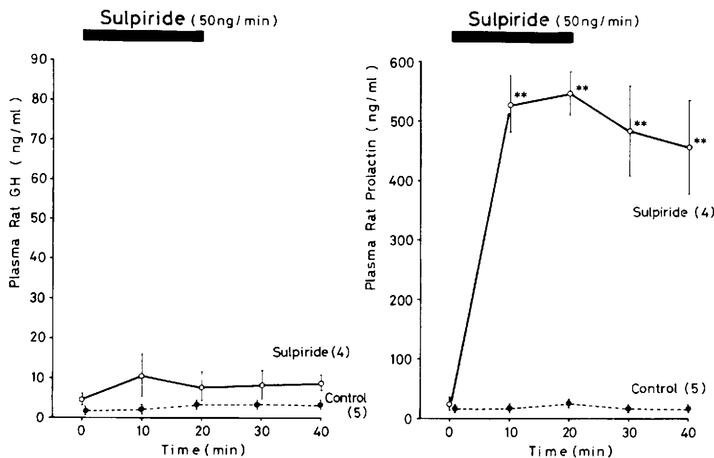

FIG. 3. Plasma GH and PRL levels following the infusion of sulpiride at a rate of 50 ng/min for 20 min into a hypophysial portal vessel in the rat. Statistical difference (vs control) is shown by asterisks: $^{**}P < 0.01$.

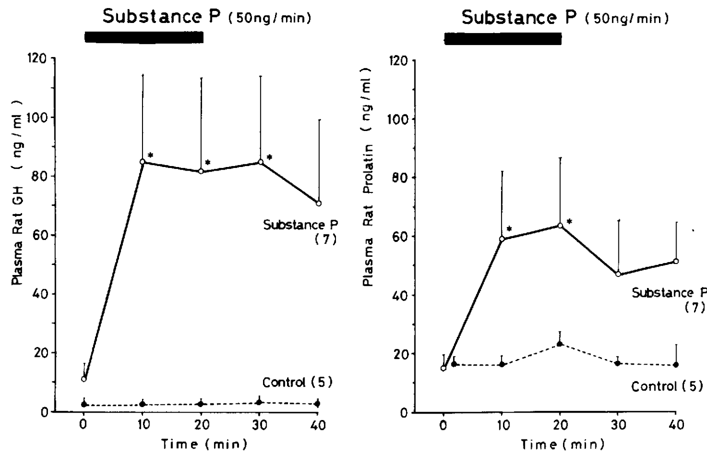

FIG. 4. Plasma GH and PRL levels following the infusion of substance P at a rate of 50 ng/min for 20 min into a hypophysial portal vessel in the rat. Statistical difference (vs control) is shown by an asterisk: $*P < 0.05$.

itary, and that this binding was competitively inhibited by such dopamine antagonists as sulpiride and haloperidol. These findings strongly suggest that dopamine has a physiological role in the inhibition of PRL secretion by acting directly, at least in part, at the pituitary level and that haloperidol and sulpiride inhibit the action of dopamine at the receptor site of the pituitary.

In the studies reported here, GH secretion was not influenced by L-dopa and dopamine antagonists infused into a hypophysial portal vessel or jugular vein. We have previously reported that in urethane-anesthetized rats, injection of chlorpromazine, a dopamine antagonist, into a jugular vein caused significant and dose-related increases in plasma GH whereas the concomitant injection of L-dopa prevented plasma GH increases induced by chlorpromazine (5). On the other

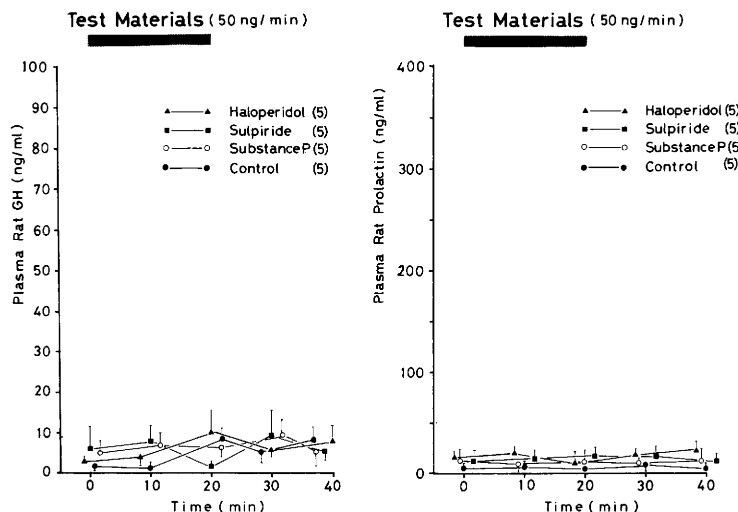

FIG. 5. Plasma GH and PRL levels following the infusion of haloperidol, sulpiride, and substance P at a rate of 50 ng/min for 20 min into the jugular vein in the rat. Phosphate-buffered saline was infused in a control group. Mean ± SE are shown. The number of animals in each test group is indicated in parentheses.

hand, the changes in GH secretion observed following L-dopa and chlorpromazine injection in intact rats were completely blunted in rats with extensive hypothalamic ablation (19, 20). We concluded, therefore, that the site of action of L-dopa and chlorpromazine in the regulation of GH release is not at the pituitary but probably in the hypothalamus. This is compatible with the present data that L-dopa and dopamine antagonists infused intraportally failed to affect GH release. Furthermore, dopamine did not affect GH release from anterior pituitary cells cultured *in vitro* (21). Therefore, it seems reasonable that effect of L-dopa treatment on GH secretion is mediated by the hypothalamus, most likely through an alteration in the secretion of a yet unidentified GH-releasing factor or somatostatin. The effect of L-dopa treatment on PRL secretion, on the other hand, is probably mediated, at least in part, by an action of dopamine directly on the pituitary gland.

We (2) have previously demonstrated that intravenous injections of substance P raised plasma GH and PRL levels in the rat. Similar results were reported by Rivier *et al.* (22). In this experiment, substance P injected into a hypophysial portal vessel caused a significant increase in GH and PRL levels, whereas it failed to change both GH and PRL levels when infused into the jugular vein in the same dose. This suggests that substance P can act directly on the pituitary gland to stimulate GH and PRL secretion. However, substance P had no effect on GH and PRL release from rat pituitary cells *in vitro* (21). Therefore, the mechanism by which substance P stimulated GH and PRL secretion remains to be elucidated.

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