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Effect of Various Catecholamine Antagonists on Prolactin Secretion in Conscious Male Rabbits (42156)

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Abstract. To clarify physiological roles of catecholaminergic systems in the control of rabbit prolactin (PRL) release, the effect of various catecholamine receptor antagonists on plasma PRL levels was examined in conscious, freely moving male rabbits. An intravenous (iv) injection of yohimbin (2.5 mg/kg body wt), an α_2-adrenoreceptor antagonist, but not prazosin (2 mg/kg body wt), an α_1-adrenergic receptor antagonist, resulted in a significant elevation of plasma PRL. Conversely, propranolol (2.5 mg/kg body wt, iv), a nonselective β-adrenoreceptor antagonist, and metoprolol (2.6 mg/kg body wt, iv), a β_1-adrenergic antagonist, slightly but significantly suppressed basal levels of plasma PRL. On the other hand, haloperidol (0.5 mg/kg body wt, iv), pimozide (0.3 mg/kg body wt, iv), sulpiride (5 mg/kg body wt, iv), chlorpromazine (3 mg/kg body wt, iv), and YM-09151-2 (0.2 mg/kg body wt, iv), all dopamine receptor antagonists caused a significant increase in plasma PRL. These results suggest that dopaminergic and α_2-adrenergic mechanisms exert a tonic inhibitory role and β-adrenergic mechanisms, probably β_1, a tonic stimulatory role in the regulation of PRL release in the rabbit.

There is considerable evidence that the central catecholaminergic system influences the secretion of prolactin (PRL) in several mammalian species (1). However, few data are available in the rabbit, probably due to delayed development of a rabbit PRL radioimmunoassay. The aim of the present study is to clarify the physiological role of the catecholaminergic system on the regulation of rabbit PRL secretion. Thus, we examined the effects of several catecholaminergic antagonists on plasma PRL levels in conscious, freely moving rabbits.

Materials and Methods. Male New Zealand White rabbits weighing 2–3 kg were provided with laboratory chow (Oriental Yeast Co., Osaka, Japan) and tap water *ad libitum* in an air-conditioned room (25 ± 1°C) under an artificial light–dark schedule (light on 0600–1800 hr). They were anesthetized with pentobarbital (25 mg/kg body wt, iv), and indwelling catheters were inserted via incision of the right external jugular vein into the right atrium at least 2 weeks before the experiment (2, 3). On the day of the experiments, a small syringe was connected to extension tubing attached to the indwelling catheter at 0900 hr; then, the animals were left undisturbed. Blood samples (0.5 ml) were collected at 15-min intervals from 1100 to 1700 hr. The test substances were injected iv as a bolus just after the first collection of blood at 1100 hr. Blood samples were centrifuged immediately, and plasma was stored at −20°C until assayed. The red blood cells were resuspended in a heparinized solution of 0.9% NaCl and returned to the rabbits after obtaining the next sample.

The following drugs were used: prazosin (Taito Pfizer Co. Ltd., Osaka, Japan), an α_1-adrenoreceptor antagonist; yohimbin (Wako Pure Chem. Industry, Osaka, Japan), an α_2-adrenoreceptor antagonist; propranolol (Sumitomo Chem. Ltd., Osaka, Japan), a β-adrenoreceptor antagonist; metoprolol (Fujisawa Pharm. Co. Ltd., Osaka, Japan), a β_1-adrenoreceptor antagonist; haloperidol (Dai-Nippon Chem. Co. Ltd., Osaka, Japan); pimozide (Fujisawa Pharm. Co. Ltd., Osaka, Japan); Chlorpromazine (Shionogi Co. Ltd., Osaka, Japan); and cis-*N*-(1-benzyl-2-methylpyrrolidin - 3 - yl) - 5 - chloro - 2 - methoxy - 4-methylaminobenzamide (YM-09151-2;

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Yamanouchi Pharm. Co. Ltd., Tokyo, Japan), a dopamine receptor antagonist. All adrenoreceptor antagonists were dissolved in distilled water, and chlorpromazine was in physiological saline. Haloperidol was dissolved in lactate, pimozide was in glacial acetic acid, and sulpiride was in sulfate. They were used after adjustment of pH to 4 with 1 *N* NaOH. YM-09151-2 was dissolved in 1 *N* HCl, diluted with distilled water, and used after adjustment of the pH to 4 with sodium bicarbonate.

Plasma PRL were measured using the radioimmunoassay (RIA) kit supplied by the National Hormone and Pituitary Agency, NIADDK, and Dr. A. F. Parlow. Highly purified rabbit PRL (AFP-1974-C) was used for radioiodination and as the reference standard. Anti-rabbit PRL serum (AFP-18102677) bound 20% of labeled rabbit PRL at a final dilution of 1:400,000. The intra- and intercoefficients of variation for the rabbit PRL RIA were 8.3 and 11.4%, respectively. The minimal detectable level of the assay was 0.03 ng/tube.

Student's *t* test was used for statistical analysis of the effect of individual dopamine receptor antagonist. Duncan's new multiple range test (4) was used for multiple comparison among the effects of adrenoreceptor antagonists.

Results. Under the baseline condition of conscious, freely moving male rabbits, plasma PRL concentrations (mean ± SEM: 5.8 ± 0.5 ng/ml) were low and stable without any significant fluctuations (Fig. 1), in sharp contrast with assay of GH showing spontaneous oscillations in an approximately 3-hr intervals as described elsewhere (2).

An intravenous injection of yohimbin, an α_2-adrenoreceptor antagonist, caused a rapid and significant increase in plasma PRL, whereas prazosin, α_1-adrenoreceptor antagonist, did not (Fig. 2).

On the contrary, an intravenous administration of metoprolol, a β_1-adrenoreceptor antagonist, as well as propranolol, a nonselective β-adrenoreceptor antagonist, resulted in a significant decrease of plasma PRL levels (Fig. 3). However, the effects of these two β-adrenoreceptor antagonists were different in duration of action; suppression of PRL release by propranolol was transient, and that by metoprolol prolonged. Dopamine receptor antagonists including sulpiride, pimozide, haloperidol, chlorpromazine, and YM-09151-

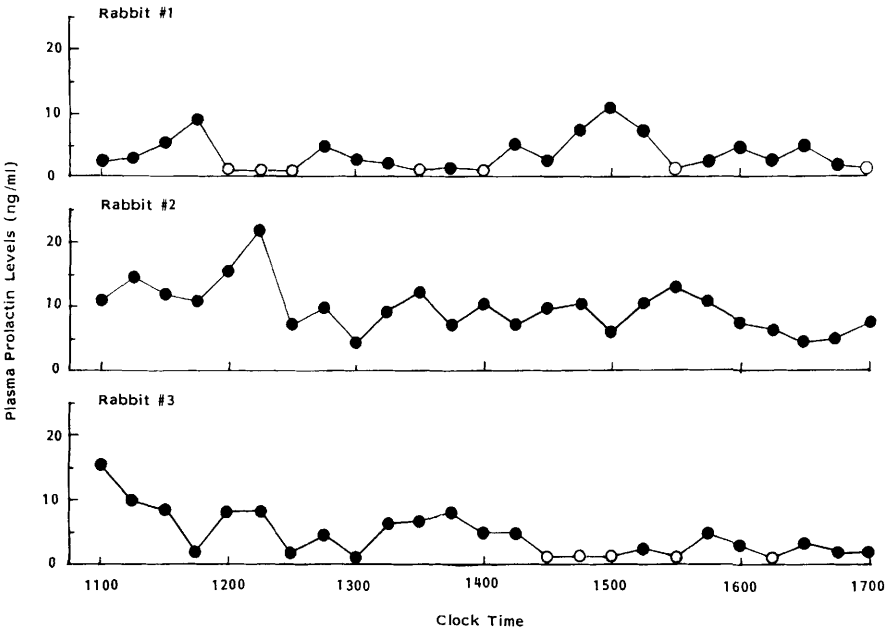

FIG. 1. Baseline prolactin secretory patterns in individual conscious, freely moving male rabbits. Open circle shows the value below the minimal detectable level in our radioimmunoassay system.

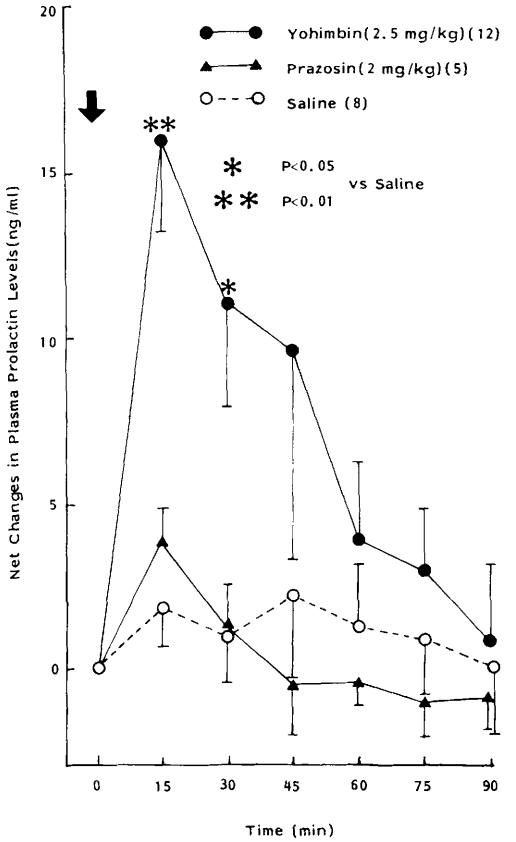

FIG. 2. Effect of yohimbin (2.5 mg/kg, iv), prazosin (2 mg/kg, iv), and saline on net changes in rabbit plasma prolactin levels. All values represent the means ± SEM from the number of determinations indicated in parentheses. Statistical difference is shown by asterisks; $*P < 0.05$, $**P < 0.01$ vs saline control (Duncan's new multiple range test).

2 were all effective in increasing plasma PRL levels (Fig. 4), although sulpiride was a D_2-selective dopamine receptor blocker, and the other four drugs were antagonists of both D_1- and D_2-dopamine receptors.

Discussion. This study showed that plasma PRL levels are affected by systemic administration of various catecholaminergic receptor antagonists, indicating an involvement of catecholaminergic mechanisms in the physiological regulation of PRL secretion in the rabbit as well as in other species.

In the present study, we found that yohimbin but not prazosin induced a marked increase in basal plasma PRL levels. On the basis of the data from the receptor binding studies, yohimbin is known to have 60–500 times greater affinity for the α_2-adrenoreceptor than prazosin (5, 6), whereas the binding affinity of prazosin to the α_1-receptor is several hundred to a thousand times greater than that of yohimbin (5, 7). Thus it is possible to assume that the basal secretion of PRL is suppressed by the tonic activation of α_2-adrenoreceptor mechanisms. Our data are consistent with the findings that yohimbin enhanced basal PRL levels in rats (8) as well as in primates (9) and stress-induced PRL release in human (10). However, the effect of clonidine, α_2-adrenoreceptor agonist, on PRL release is controversial. Gold *et al.* have reported an inhibitory effect of clonidine (10 μg/kg, iv) on PRL release in nonhuman primates (11), but Negro-vilar *et al.* have demonstrated the stimulatory effect of clonidine (150 μg/kg, iv) on PRL release in ovariectomized rats (12). Our data rather supported the former results, although the cause of the discrepancy remains unknown.

A single iv bolus injection of propranolol caused a small but significant decrease in

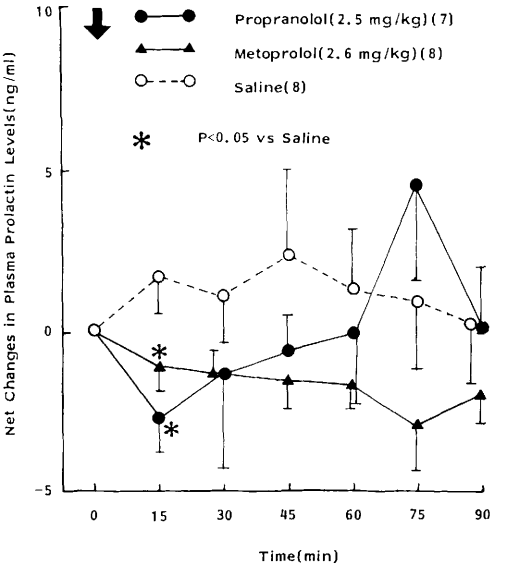

FIG. 3. Effect of propranolol (2.5 mg/kg, iv), metoprolol (2.6 mg/kg, iv), and saline on net changes in rabbit plasma prolactin levels. All values represent the means ± SEM from the number of determinations indicated in parentheses. Statistical difference is shown by asterisks; $*P < 0.05$ vs saline control (Duncan's new multiple range test).

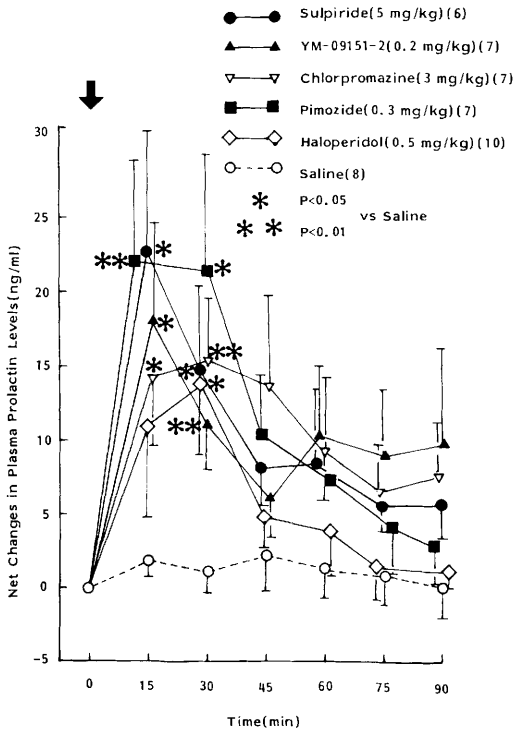

FIG. 4. Effect of sulpiride (5 mg/kg, iv), YM-09151-2 (0.2 mg/kg, iv), chlorpromazine (3 mg/kg, iv), pimozide (0.3 mg/kg, iv), haloperidol (0.5 mg/kg, iv), and saline on net changes in rabbit plasma prolactin levels. All values represent the means ± SEM from the number of determinations indicated in parentheses. Statistical difference is shown by asterisks; $*P < 0.05$, $**P < 0.01$ vs saline control (Student's t test).

plasma PRL in the present study, which is in good agreement with the previous data in conscious male rats (13). However, the effect of propranolol on PRL secretion is inconsistent and different under various experimental conditions. Gala *et al.* have reported that plasma PRL was increased modestly by propranolol in monkeys at a dose of 5 mg/kg, iv (14) and in ovariectomized, estrogen-treated rats at a dose of 50 mg/kg iv (15) and was enhanced markedly by intracerebroventricular injection of propranolol (50 μg/5 μl) in female rats (16). Wartofsky *et al.* have reported that propranolol had no effect on either basal plasma PRL levels or TRH-induced PRL release in humans (17). The reason the effect of propranolol on PRL release is inconsistent among different experimental conditions remains unknown. However, this discrepancy might be due to the failure of propranolol to select the subclass of β-adrenoreceptors. In this study, we found that an iv injection of metoprolol, a β_1-adrenoreceptor antagonist, caused a modest, sustained decrease in plasma PRL throughout the experiment, while plasma PRL levels declined only transiently after propranolol, then returned to the basal value and rather tended to increase 75 min after the injection. Although the role of the β_2-adrenoreceptor mechanism in controlling PRL secretion remains to be investigated, there might be a reciprocal relationship between β_1- and β_2-adrenoreceptors in the regulation of PRL release.

On the other hand, dopamine is well established as one of the PRL release-inhibiting factors acting through hypophyseal portal circulation to suppress directly PRL release from the anterior pituitary gland, whereas D_2-, but not D_1-, dopaminergic receptors have been reported to exist (18, 19). Our observation on stimulation of PRL release by sulpiride and chlorpromazine agrees well with previous findings obtained from the rabbit (20, 21) and other species (1). YM-09151-2, a novel benzamide, was initially reported to antagonize the action of dopamine through D_1-dopaminergic receptors (22), while it is now considered to be a rather potent antagonist of D_2-type receptors (23, 24). YM-09151-2, pimozide, and haloperidol also caused a prompt and significant increase of plasma PRL in the rabbit in our study, in concordance with their stimulatory effects on PRL release in other species (1, 24). Thus, D_2-dopaminergic receptor mechanisms appear to play a tonic inhibitory role in regulating rabbit PRL release.

In conclusion, the catecholaminergic regulation of PRL release in the rabbit may be accomplished by the tonic inhibition via both α_2-adrenoreceptor and D_2-dopamine receptor mechanisms as well as by tonic stimulation via β_1-adrenoreceptor mechanisms. The site of receptors involved in PRL secretion cannot be determined by this study.

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