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Inhibitory Effects of Putative Dopamine D_3 Receptor Agonists, 7-OH-DPAT and Quinpirole, on Prolactin Secretion in Rats

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KURASHIMA, M., M. DOMAE, T. INOUE, M. NAGASHIMA, K. YAMADA, K. SHIRAKAWA AND T. FURUKAWA. *Inhibitory effects of putative dopamine D_3 receptor agonists, 7-OH-DPAT and quinpirole, on prolactin secretion in rats.* PHARMACOL BIOCHEM BEHAV **53**(2) 379–383, 1996.—The present experiments were performed to investigate effects of (±)-2-(dipropylamino)-7-hydroxy-1,2,3,4-tetrahydronaphthalene (7-OH-DPAT) or quinpirole (LY 171555), putative dopamine (DA) D_3 receptor agonists, on serum prolactin levels in male rats. Basal prolactin levels were reduced dose-dependently by SC administration of 7-OH-DPAT or quinpirole at respective doses of 10–100 μg/kg and 25–250 μg/kg. Daily treatment with estradiol, 35 μg/kg/day for 3 days, increased serum prolactin levels to fourfold higher levels than those of nonprimed rats. Intraperitoneal injection of α-methyl-p-tyrosine (α-MT), 300 mg/kg, also increased serum prolactin levels. 7-OH-DPAT or quinpirole at a dose of 50 μg/kg caused a marked reduction in serum prolactin levels in both the estradiol- and α-MT-induced hyperprolactinemia. The 7-OH-DPAT- and quinpirole-induced decreases in serum prolactin levels were antagonized by the administration of the DA D_2 receptor antagonist, spiperone, at 0.5 mg/kg. The results indicate that 7-OH-DPAT and quinpirole decrease prolactin levels in rats by stimulation of the D_2 receptor.

Prolactin 7-OH-DPAT Quinpirole 17β-Estradiol α-Methyl-p-tyrosine Rat

BECAUSE the mode of action of antiparkinsonian and antipsychotic drugs has been proposed to involve interaction with dopamine (DA) receptors, much attention has been paid to the characterization of the precise targets of these drugs in the brain (14). DA in the central nervous system is also involved in regulation of hormone secretion. Tuberoinfundibular DA neurons, which originate in the arcuate and periventricular nuclei of the hypothalamus and project to the external layer of the median eminence, are known to regulate prolactin secretion from the anterior pituitary. DA released from the median eminence into the hypophyseal portal vessels reaches the anterior pituitary and tonically suppresses prolactin secretion by acting on D_2 receptors on prolactin-secreting cells (1).

DA receptors were originally classified as D_1 and D_2 based on their abilities to stimulate or inhibit the adenylate cyclase activity (6). The potency order of antipsychotic drugs is known to be correlated with binding affinities for D_2 receptors (12). However, biological and pharmacological evidence indicates that the original D_2 receptors could consist of similar but discrete receptors (13,17). The cloning and characterization of several cDNAs for DA receptors have shown that a DA receptor family consists of two subclasses, e.g., the D_1-like (D_1 and D_5) receptors, and the D_2-like (D_2, D_3, and D_4) receptors (15). In comparison of anatomical distributions between D_2 and D_3 receptor mRNAs in the brain, D_2 receptor mRNAs are most abundantly found in the dopaminoceptive regions and dopamine-containing cells, whereas D_3 receptor mRNAs are found in primarily in the limbic area, such as the accumbens nuclei, islands of Calleja, and bed nucleus of the stria terminalis (2). Many antipsychotics display very high affinities for the D_3

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receptor expressed in Chinese hamster ovary cells (17). Therefore, the D_3 receptor has been investigated as a target for antipsychotics.

Although most of D_2 receptor agonists and antagonists do not discriminate between the D_2 and D_3 receptors, (±)-2-(dipropylamino)-7-hydroxy-1,2,3,4-tetrahydronaphthalene (7-OH-DPAT) and quinpirole (LY 171555) have been reported to recognize D_3 receptors with approximately 100-fold higher affinities than D_2 receptors in competition experiments using transfected receptors, in which [^{3}H]7-OH-DPAT binding saturably occurs at the D_3 receptor but not at the D_2 receptor (8). According to the recent proposal, in our previous behavioral experiments, we investigated effects of the putative dopamine D_3 receptor agonists, 7-OH-DPAT and quinpirole, on yawning, stereotypy, and body temperature in rats (7).

In this experiment, we studied effects of 7-OH-DPAT and quinpirole, on serum prolactin levels in rats.

METHOD

Animals

Male Wistar rats (200–350 g) were obtained from Kyudo Animal Laboratory (Kumamoto, Japan). They were kept in an animal room with a 12 L : 12 D cycle (lights on at 0700 h). Commercial food (CE-2, Clea Ltd., Osaka, Japan) and tap water were freely available except during the experiments.

Administration of Drugs

7-OH-DPAT (10–100 μg/kg) and quinpirole (25–250 μg/kg) were administered subcutaneously (SC) 30 min prior to decapitation. α-Methyl-p-tyrosine (α-MT) (300 mg/kg) was given intraperitoneally (IP) as a suspension in 0.5% carboxymethylcellulose 1 h before sacrifice. Male rats received SC injections of estradiol (35 μg/kg/day) dissolved in sesame oil for 3 consecutive days, and control animals received SC injections of vehicle, the last treatment being 24 h before killing. Spiperone (0.5 mg/kg, SC) was injected 60 min before sacrifice. These drug dosages were selected according to our previous experiments (4,9).

Determination of Prolactin Concentrations

Blood samples were collected from the trunks of decapitated rats and were centrifuged at 3000 × *g* and 4°C for 30 min. Serum prolactin levels were determined by a double-antibody radioimmunoassay as previously described (4). The reagents were kindly supplied from the National Hormone and Pituitary Agency (rat prolactin RP-3 standard and antirat prolactin serum-9). Each sample was assayed in duplicate at several dilutions.

Drugs

The following drugs were used: (±)-2-(dipropylamino)-7-hydroxy-1,2,3,4-tetrahydronaphthalene (7-OH-DPAT) hydrobromide (MW : 328.3), quinpirole (LY 171555) hydrochloride (MW : 255.8) (Research Biochemicals Inc., Natick, MA), DL-α-methyl-p-tyrosine (α-MT) (MW : 195.2) (Nacalai tesque, Kyoto, Japan), 17β-estradiol 3-benzoate (MW : 376.5) (Sigma, St. Louis, MO). 7-OH-DPAT and quinpirole were dissolved in saline. Doses were expressed in terms of salt except for α-MT.

Statistical Analysis

Results are expressed as means ± SEM. Statistical analysis was done using a one-way analysis of variance followed by the two-tailed Dunnett's test for difference between a control and all means or Tukey test for differences between all means (19).

RESULTS

Inhibitory Effects of 7-OH-DPAT and Quinpirole on Basal Serum Prolactin Levels

The mean serum prolactin level in the saline-injected rats was 9.4 ± 1.0 ng/ml. Serum prolactin levels were diminished in a dose-dependent manner by administration of 7-OH-DPAT at doses ranging from 10 to 100 μg/kg (Fig. 1). Quinpirole also decreased serum prolactin levels in a dose-dependent manner at 25–250 μg/kg. The effects of 7-OH-DPAT appeared to be a little more potent than those of quinpirole.

Inhibitory Effects of 7-OH-DPAT and Quinpirole on the Hyperprolactinemia Induced by Estradiol

17β-Estradiol increased serum prolactin levels by about fourfold above the basal prolactin level (44.3 ± 6.5 ng/ml, n = 10). The estradiol-induced hyperprolactinemia was strongly suppressed by the administration of 7-OH-DPAT at 50 μg/kg, the prolactin level being reduced to 22.4% of the control group treated with estradiol plus saline (Fig. 2). The hyperprolactinemia was similarly suppressed to 26.2% of the control group by quinpirole at 50 μg/kg.

Inhibitory Effects of 7-OH-DPAT and Quinpirole on the Hyperprolactinemia Induced by α-MT

Administration of α-MT (300 mg/kg, IP) markedly increased serum prolactin levels, the levels in saline plus saline-injected group and α-MT plus saline-injected group being 11.4 ± 1.1 and 39.4 ± 8.6 ng/ml, respectively. As shown in Fig. 3, the α-MT-induced hyperprolactinemia was markedly suppressed in a similar degree by 7-OH-DPAT and quinpirole.

Antagonism by Spiperone of 7-OH-DPAT- and Quinpirole-Induced Decreases in Serum Prolactin Levels

Spiperone (0.5 mg/kg, SC), a DA D_2 receptor antagonist, increased serum prolactin levels by about sixfold above the basal prolactin level, implying that the agent competes with endogenous DA from the median eminence at D_2 receptors located on prolactin secreting cells. The basal prolactin levels declined following the injection of 7-OH-DPAT or quinpirole at 50 μg/kg. The 7-OH-DPAT- and quinpirole-induced decreases in serum prolactin levels were antagonized by treatment with spiperone (Fig. 4).

DISCUSSION

Prolactin-secreting cells are endowed with inhibitory D_2 receptors that are encoded by the same gene found in the brain (3). DA released from the median eminence of the hypothalamus is a major regulatory neurotransmitter on secretion of prolactin (1). We also confirmed that, in in vitro experiments on isolated rat anterior pituitary slices, spontaneous prolactin release was decreased by the D_2 receptor agonists, talipexole and SND 919, and that the inhibitory effects of these drugs were antagonized by the D_2 receptor antagonist, YM-09151-2 (4). In addition, we showed that talipexole and SND 919 decreased serum prolactin levels in a dose-dependent manner (5–100 μg/kg) in rats (4,9). Quinpirole was also reported to decrease release of prolactin from cultured anterior pituitary cells (10,18). In the present experiments, the putative DA D_3

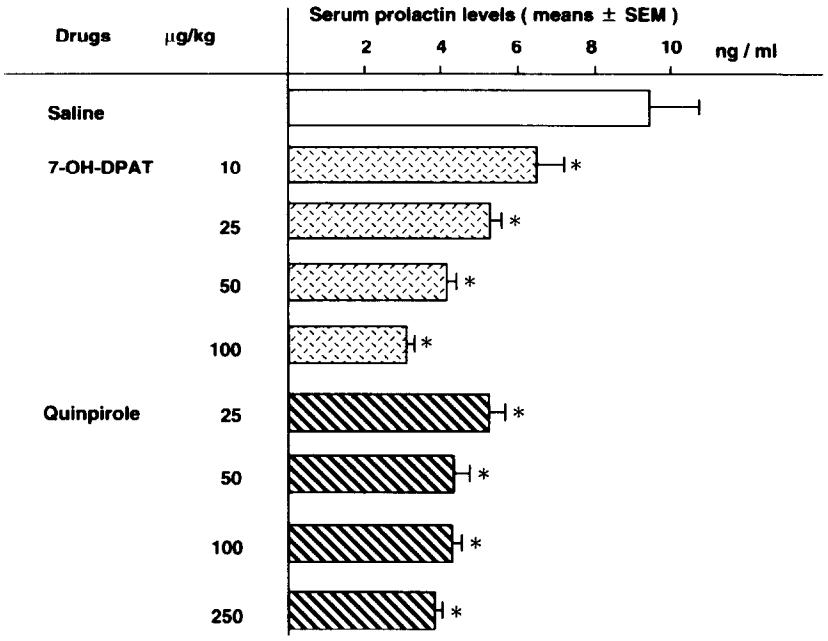

FIG. 1. Inhibitory effects of 7-OH-DPAT and quinpirole on basal serum prolactin levels. Drugs were subcutaneously injected 30 min prior to blood sampling. Horizontal bars represent the means ± SEM of serum prolactin levels from 10 rats. $*p < 0.01$; significant difference from the saline-injected group.

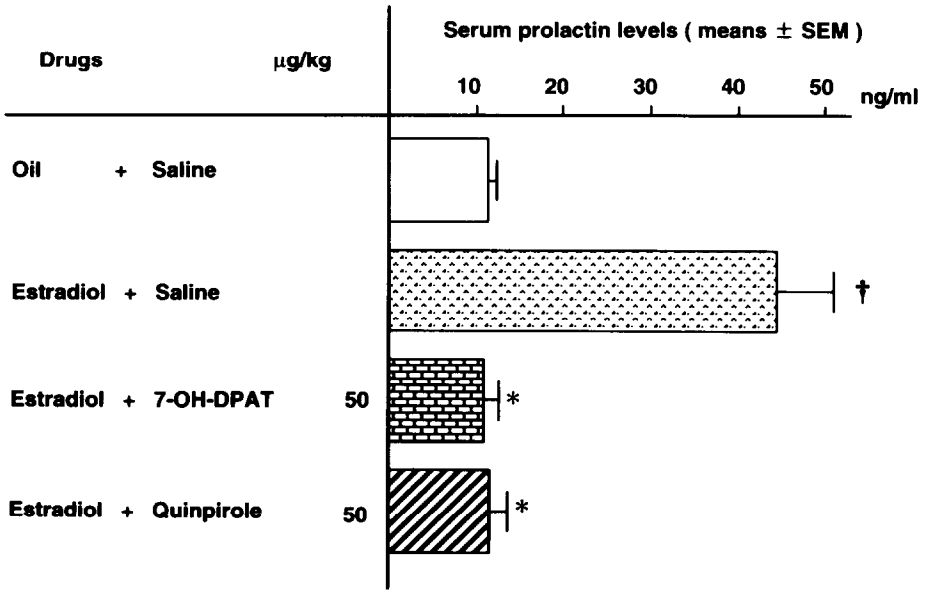

FIG. 2. Inhibitory effects of 7-OH-DPAT and quinpirole on the hyperprolactinemia induced by estradiol. 17β-Estradiol, 35 μg/kg per day, was subcutaneously given for 3 consecutive days. Saline or drugs were administered 30 min before blood sampling. Horizontal bars represent the means ± SEM of serum prolactin levels from 10 rats. $†p < 0.01$; significant difference from the oil plus saline-injected group. $*p < 0.01$; significant difference from the estradiol plus saline-injected group.

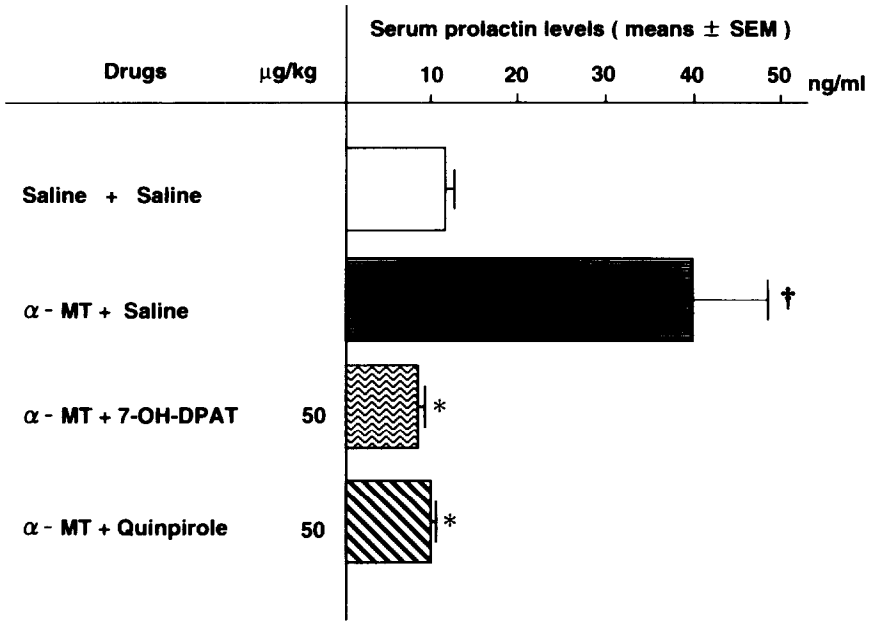

FIG. 3. Inhibitory effects of 7-OH-DPAT and quinpirole on the hyperprolactinemia induced by α-MT. α-MT (300 mg/kg) was intraperitoneally injected 1 h prior to blood sampling. Saline or drugs were administered 30 min before blood sampling. Horizontal bars represent the means ± SEM of serum prolactin levels from 10 rats. †$p < 0.01$; significant difference from the saline-injected group. *$p < 0.01$; significant difference from the α-MT plus saline-injected group.

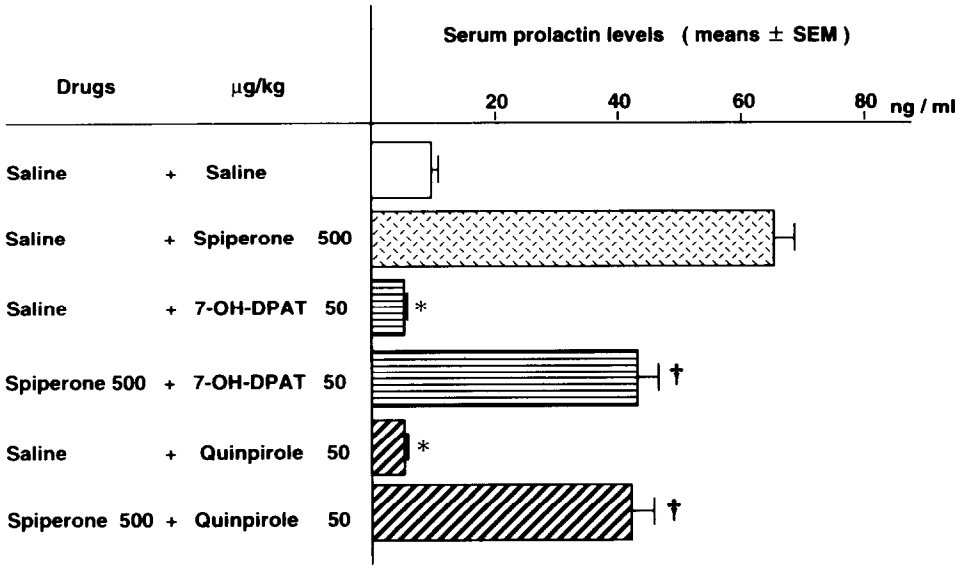

FIG. 4. Antagonism by spiperone of 7-OH-DPAT- and quinpirole-induced decreases in serum prolactin levels. Spiperone was subcutaneously administered 60 min, and saline, 7-OH-DPAT, or quinpirole 30 min, prior to blood sampling. Horizontal bars represent means ± SEM of serum prolactin levels from 10 rats. *$p < 0.01$; significant difference from the saline plus saline-injected group. †$p < 0.01$; significant difference from the saline plus respective agonist-injected group.

receptor agonists, 7-OH-DPAT and quinpirole, decreased serum prolactin levels in a dose-dependent manner (10–250 μg/kg). This dose range is quite similar to that of talipexole or SND 919, D_2 receptor agonists. Accordingly, the putative DA D_3 receptor agonists seem to effectively recognize D_2 and/or D_3 receptors and thereby decrease prolactin secretion. α-MT, an inhibitor of tyrosine hydroxylase, blocks synthesis of DA in the nerve terminals of the median eminence and decreases concentrations of DA in the hypophyseal portal vessel (1,5). Treatment with estradiol is well known to increase synthesis and storage of prolactin (1). Our previous studies showed that the α-MT- and estradiol-induced hyperprolactinemia was markedly reduced by talipexole or SND 919 (5–100 μg/kg, SC) (4,9). In this study, the α-MT- and estradiol-induced hyperprolactinemia was similarly inhibited by 7-OH-DPAT or quinpirole (50 μg/kg), indicating that the putative D_3 receptor agonists decrease prolactin levels at similar dose ranges for D_2 receptor agonists.

The anterior pituitary is found to be rich in D_2 receptors, but lacks D_3 receptors (16). 7-OH-DPAT was reported to have higher affinity for D_3 receptors than for D_2 receptors in inhibiting [^{125}I]iodosulpride-binding to membranes from Chinese hamster ovary cells expressing each receptor, with respective dissociation constant (K*i*) values of 0.78 ± 0.02 and 61 ± 2 nM (8). Under the same experimental conditions, the D_2 receptor has low affinity for DA with K_i value of 474 ± 33 nM (8,16). Interestingly, it has been reported that 7-OH-DPAT and quinpirole have similar potencies for D_2 and D_3 receptors transfected in frog melanophores in causing pigment aggregation (11). In fact, 7-OH-DPAT- and quinpirole-produced decreases in serum prolactin levels were antagonized by spiperone, a dopamine D_2 receptor antagonist, in these studies. Accordingly, 7-OH-DPAT and quinpirole probably interact with D_2 receptors on prolactin-secreting cells in in vivo experiments.

The results show that putative D_3 receptor agonists, 7-OH-DPAT and quinpirole, decrease serum prolactin levels, as do D_2 receptor agonists, in rats.

REFERENCES

1. Ben-Jonathan, N. Dopamine: A prolactin-inhibiting hormone. Endocr. Rev. 6:564–589; 1985.
2. Bouthenet, M.-L.; Souil, E.; Martres, M.-P.; Sokoloff, P.; Giros, B.; Schwartz, J.-C. Localization of dopamine D_3 receptor mRNA in the rat brain using in situ hybridization histochemistry: Comparison with dopamine D_2 receptor mRNA. Brain Res. 564: 203–219; 1991.
3. Bunzow, J. R.; Van Tol, H. H. M.; Grandy, D. K.; Albert, P.; Salon, J.; Christie, M.; Machida, C. A.; Neve, K. A.; Civelli, O. Cloning and expression of a rat D_2 dopamine receptor cDNA. Nature 336:783–787; 1988.
4. Domae, M.; Yamada, K.; Hanabusa, Y.; Matsumoto, S.-I.; Furukawa, T. Decrease of prolactin secretion via stimulation of pituitary dopamine D_2 receptors after application of talipexole and SND 919. Eur. J. Pharmacol. 179:75–82; 1990.
5. Greef, W. J.; Neill, J. D. Dopamine levels in hypophysial stalk plasma of the rat during surges of prolactin secretion induced by cervical stimulation. Endocrinology 105:1093–1099; 1979.
6. Kebabian, J. W.; Calne, D. B. Multiple receptors for dopamine. Nature 277:93–96; 1979.
7. Kurashima, M.; Yamada, K.; Nagashima, M.; Shirakawa, K.; Furukawa, T. Effects of putative dopamine D-3 receptor agonists, 7-OH-DPAT and quinpirole, on yawning, stereotypy and body temperature in rats. Pharmacol. Biochem. Behav. (in press).
8. Lévesque, D.; Diaz, J.; Pilon, C.; Martres, M.-P.; Giros, B.; Souil, E.; Schott, D.; Morgat, J.-L.; Schwartz, J.-C.; Sokoloff, P. Identification, characterization, and localization of the dopamine D_3 receptor in rat brain using 7-[^{3}H]hydroxy-N,N-di-n-propyl-2-aminotetralin. Proc. Natl. Acad. Sci. USA 89:8155–8159; 1992.
9. Matsumoto, S.-I.; Yamada, K.; Nagashima, M.; Domae, M.; Shirakawa, K.; Furukawa, T. Occurrence of yawning and decrease of prolactin levels via stimulation of dopamine D_2-receptors after administration of SND 919 in rats. Naunyn Schmiedeberg's Arch. Pharmacol. 340:21–25; 1989.
10. Meller, E.; Puza, T.; Diamond, J.; Lieu, H.-D.; Bohmaker, K. Comparative effects of receptor inactivation, 17β-estradiol and pertussis toxin on dopaminergic inhibition of prolactin secretion in vitro. J. Pharmacol. Exp. Ther. 263:462–469; 1992.
11. Potenza, M. N.; Graminski, G. F.; Schmauss, C.; Lerner, M. R. Functional expression and characterization of human D_2 and D_3 dopamine receptors. J. Neurosci. 14:1463–1476; 1994.
12. Seeman, P.; Lee, T.; Chau-Wong, M.; Wong, K. Antipsychotic drug doses and neuroleptic/dopamine receptors. Nature 261:717–718; 1976.
13. Seeman, P.; Schaus, J. M. Dopamine receptors labelled by [^{3}H] quinpirole. Eur. J. Pharmacol. 203:105–109; 1991.
14. Seeman, P.; Van Tol, H. H. M. Dopamine receptor pharmacology. Trends Pharmacol. Sci. 15:264–270; 1994.
15. Sibley, D. R.; Monsma, F. J., Jr. Molecular biology of dopamine receptors. Trends Pharmacol. Sci. 13:61–69; 1992.
16. Sokoloff, P.; Giros, B.; Martres, M.-P.; Bouthenet, M.-L.; Schwartz, J.-C. Molecular cloning and characterization of a novel dopamine receptor (D_3) as a target for neuroleptics. Nature 347: 146–151; 1990.
17. Sokoloff, P.; Martres M. P.; Schwartz, J. C. Three classes of dopamine receptor (D_2, D_3, D_4) identified by binding studies with ^{3}H-apomorphine and ^{3}H-domperidone. Naunyn Schmiedeberg's Arch. Pharmacol. 315:89–102; 1980.
18. Tagawa, M.; Takahara, J.; Sato, M.; Niimi, M.; Murano, K.; Ishida, T. Stimulatory effects of quinpirole hydrochloride, D_2-dopamine receptor agonist, at low concentrations on prolactin release in female rat in vitro. Life Sci. 51:727–732; 1992.
19. Winer, B. J. Statistical principles in experimental design. 2nd ed. Tokyo: McGraw-Hill; 1971.