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Binding Properties of Higenamine on Dopamine Receptors of Caudate Nucleus in Bovine Brain†

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= Abstract = The binding properties of higenamine on dopamine receptors in bovine brain caudate nucleus membrane preparations were studied by means of inhibition of ^{3}H-dopamine binding. The binding of ^{3}H-dopamine to D-2 receptor was inhibited by much lower concentration of higenamine(nanomolar range) than that to D-1 receptor(micromolar range), and the Hill coefficient for higenamine inhibition of ^{3}H-dopamine binding was 0.91 while those for sulpiride and metoclopramide inhibition were 0.22 and 0.59, respectively. These results suggested that higenamine would have agonistic activity on dopamine receptors with higher affinity for D-2 receptor than for D-1 receptor.

Key Words: ***Binding study, Higenamine, ^{3}H-dopamine, Bovine caudate nucleus, D-1, D-2 receptor***

INTRODUCTION

Aconiti tuber which belongs to Ranunculaceae family plant has long been used in oriental medicine as cardiotonic, diuretic and analgesic(Park and Kim 1981). Higenamine which has strong cardiotonic action was found in this plant along with other well known alkaloids such as aconitine. Our previous studies(Park *et al.* 1984) in excised auricles demonstrated that higenamine has a potent positive inotropic effect which was blocked by propranolol. It was also found that its positive inotropic effect was potentiated by calcium whereas the depressant effect of calcium antagonists such as verapamil or lanthanum on the contractile force of heart was reversed by higenamine(Chang *et al.* 1981).

These results suggested that the inotropic action of higenamine may be mediated through cardiac adrenoceptor stimulation by higenamine. However, the presence of dopamine moiety in the structure of higenamine does not exclude the possibility of involvement of dopaminergic mechanism in the inotropic action of this agent. To investigate this possibility of its dopaminergic effect, the binding properties of higenamine on dopamine receptors of bovine caudate nucleus were examined through comparison with those of known dopaminergic agonists and antagonists.

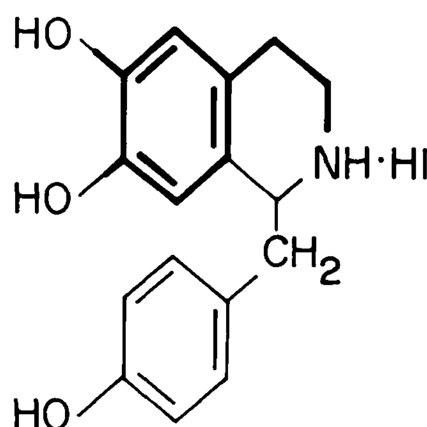

Fig. 1. Chemical structure of higenamine. Dopamine moiety is indicated by bold line.

METHODS

Crude synaptosomal membranes were prepared according to List and Seeman(1980) with slight modifications. The caudate nucleus of bovine brain, fresh from a slaughterhouse, was dissected and homogenized with Brinkmann Polytron PCU-I in 15 volumes of cold 15 mM Tris-HCl buffer, pH 7.4. The homogenate was centrifuged for 20 min at

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900×g after incubation for 30 min at 37°C. The resulting supernatant was centrifuged for 30 min at 35,000×g. The pellet obtained was washed with cold TEAP buffer(15 mM Tris-HCl, 5 mM Na_2 EDTA, 1.1 mM ascorbic acid and 12.5 μM pargyline, pH 7.4) three times in order to eliminate endogenous dopamine. The final suspension in TEAP buffer was frozen at −20°C until assayed. The protein content of the tissue preparation was determined by the method of Lowry *et al.*(1951).

Before each assay, the frozen membrane preparation was subjected to Polytron at rheostat setting at 7 for 10 sec. Binding assay was performed separately for D-1 and D-2 receptor according to the method of Nishikori *et al.*(1980). The standard assay tubes received 100 μl of diluted ^{3}H--dopamine(final concentration of 5 μM for D-1 receptor and 5 nM for D-2 receptor, respectively), 200 μl of various concentrations of non-radioactive ligands and 200 μl of membrane suspension(1 mg protein of tissue preparation). After incubation in the water bath shutted from light at 22°C for 30 min, each reaction mixture was quickly filtered through Whatman GF/B filter in vacuo followed by washing twice with 5 ml of ice-cold TEAP buffer. Blotted filters were shaken vigorously in a counting vial with 6 ml of scintillation cocktail for 15 min. The radioactivity for ^{3}H was monitored with Beckman LS 8800 after standing over 6 hours at 4°C to allow temperature equilibration and homogenous translucency of GF/B filter. The specific binding of ^{3}H-dopamine was defined as that removed by adding an excess of non-radioactive dopamine(1 mM for D-1 receptor and 1 μM for D-2 receptor). The concentration of inhibitor producing 50 per cent inhibition of ^{3}H-dopamine specific binding(IC50) was calculated by logit-log analysis(De Lean *et al.* 1978). The inhibition constant (Ki) of tested drug was calculated from the following equation by the method of Cheng and Prusoff (1973);

$$Ki = IC50(1 + (D)/kd)$$

where Kd=dissociation constant of ^{3}H-dopamine derived from Scatchard analysis, (D)=concentration of ^{3}H-dopamine.

The radioactive ^{3}H-dopamine was obtained from New England Nucear(24.5-30.4 Ci/m mol). Higenamine was kindly synthesized by Dr. H.S. Yun, Natural Products Research Institute, Seoul National University. Bromocriptine mesylate, sulpiride and metoclopramide were obtained gratis from Dong-Wha, Dae-Woong, and Dong-A Pharmaceutical Co. Ltd. Korea, respectively.

Bromocriptine and sulpiride were dissolved in small amount of 1% (V/V) and 2% sulfuric acid, respectively followed by diluted to required concentrations and adjusted to pH 7.4 with dilute NaOH solution.

RESULTS

1 Specific ^{3}H-dopamine binding on dopamine receptors

The binding of ^{3}H-dopamine to dopamine receptors in the membrane preparation of bovine caudate nucleus was saturable as shown in Fig. 2 and 3. Scatchard analysis of ^{3}H-dopamine binding to D-1 and D-2 receptor showed dissociation constant(Kd) of 8.1+0.7 μm with Bmax of 116.5+5.9 pmol/mg protein for D-1 receptor and Kd of 7.9+0.6 nM with Bmax of 374.4+8.8 fmol/mg protein for D-2 receptor.

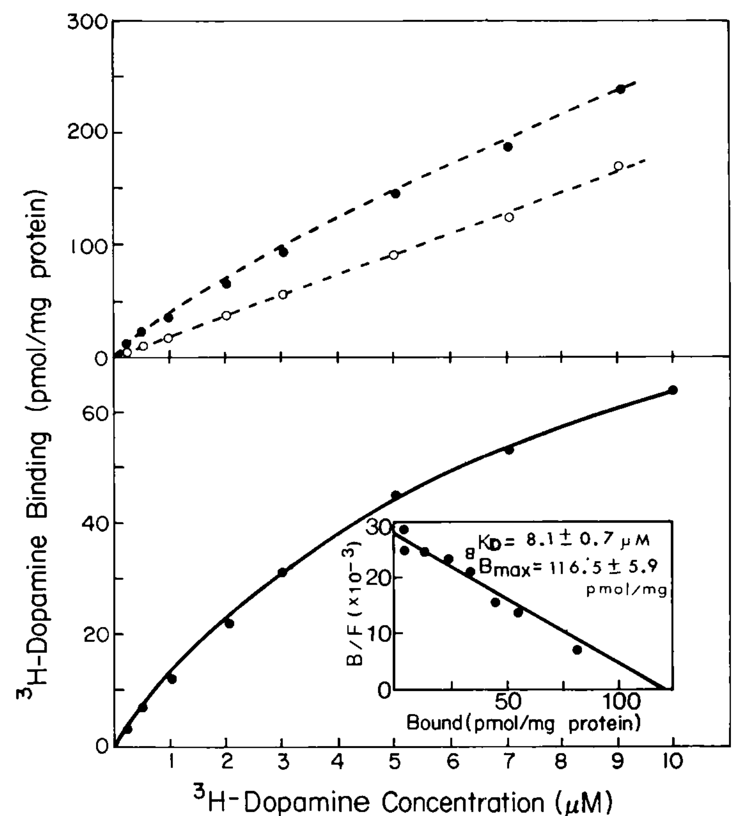

Fig. 2. Saturability of specific ^{3}H-dopamine binding to D-1 receptor of bovine caudate nucleus membrane. Specific ^{3}H-dopamine binding(●) to D-1 receptor(lower panel) was determined as the difference between the binding in the absence(●) and presence(○) of 1 mM cold dopamine(upper panel). Inset in the lower panel is Scatchard plot of ^{3}H-dopamine binding to D-1 receptor. B/F: the ratio of bound to free ^{3}H-dopamine, Kd: dissociation constant, Bmax: maximum binding.

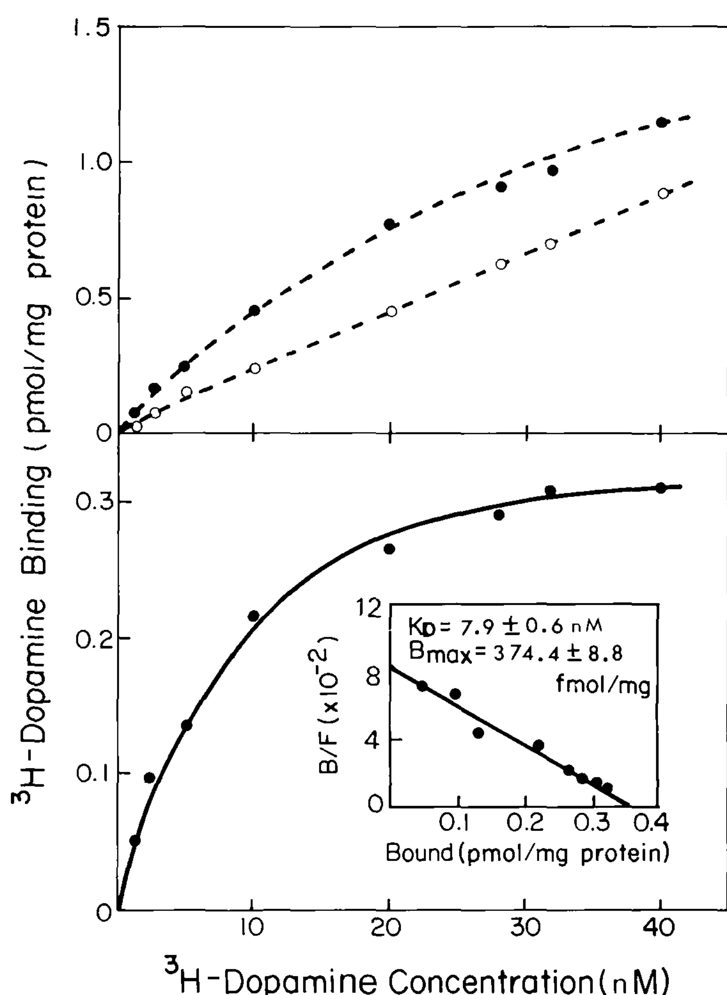

Fig. 3. Saturability of specific ^{3}H-dopamine binding to D-2 receptor of bovine caudate nucleus membrane. Specific ^{3}H-dopamine binding(●) to D-2 receptor was determined as in the legend to Fig. 2 except 1 μM cold dopamine instead of 1 mM cold dopamine.

2 Effects of higenamine on ^{3}H-dopamine binding

In bovine caudate nucleus membranes, higenamine competed with ^{3}H-dopamine for binding to both D-1 and D-2 receptors(Fig. 4,6) but the

Table 1. Ki values for ^{3}H-dopamine binding to D-1 and D-2 receptors(Mean ± S.E.)

Drugs	Ki for ^{3}H-dopamine binding	
	D-1	D-2
	μM	μM
Higenamine	15.85±1.25	0.144 ±0.010
Dopamine	7.00±0.85	0.0096±0.0007
Apomorphine	31.50±1.04	0.0497±0.0027
Bromocriptine	25.45±0.85	0.282 ±0.032
Sulpiride	100	1.73 ±0.24
Metoclopramide	100	57.55 ±4.45

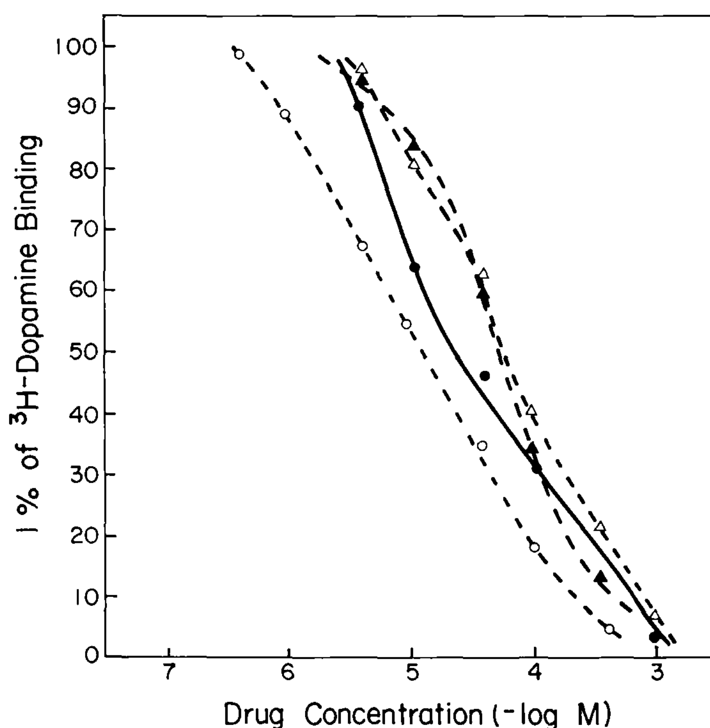

Fig. 4. Effects of higenamine and various ligands on the binding of ^{3}H-dopamine to D-1 receptor. Increasing concentrations of higenamine(●), non-radioactive dopamine(○), apomorphine(△) and *bromocriptine*(▲) were added to tubes containing 5 μM ^{3}H-dopamine and bovine caudate nucleus membrane preparation equivalent to 5 mg protein per ml.

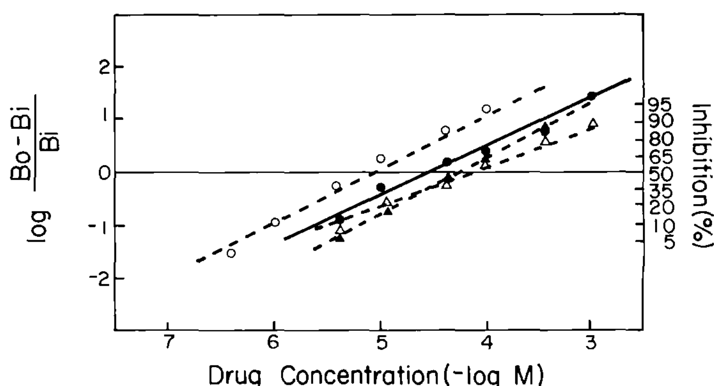

Fig. 5. *Logit-log inhibition plot(Hill plot) of* ^{3}H-dopamine binding to D-1 receptor. Data are from the same experiment as Fig. 4. Bo: Specific binding of ^{3}H-dopamine in the absence of the drugs, Bi: specific binding of ^{4}H-dopamine in the presence of the drugs.

potency of higenamine to displace the ^{3}H-dopamine for D-2 receptor(Ki=144 nM) was hundred times greater than that for D-1 receptor(Ki=15.85 μM)(Table 1).

Higenamine inhibited ^{3}H-dopamine binding 50% for D-2 receptor at 356 nM concentration while

concentrations for 50% inhibition (IC50) of binding for dopamine itself, bromocriptine and apomorphine were 24 nM, 123 nM and 697 nM respectively. The slopes of Hill plot for inhibition of bindings to D-1 and D-2 receptor by higenamine, dopamine, apomorphine and bromocriptine were approximately parallel but sulpiride and metoclopramide showed smaller values than those of other ligands tested(Fig. 5,7).

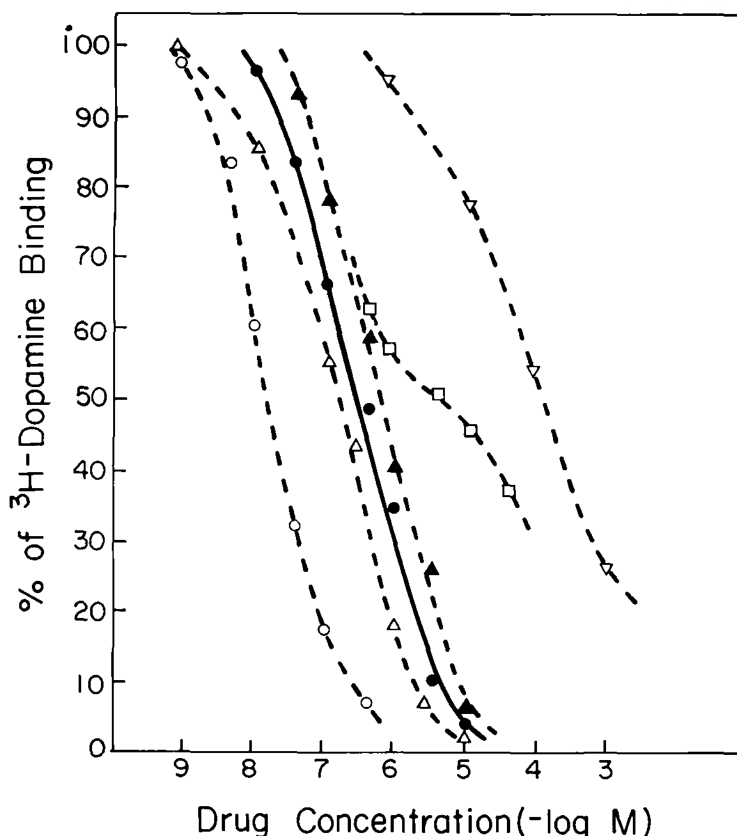

Fig. 6. Effects of higenamine and various ligands on the binding of ^{3}H-dopamine to D-2 receptor. Increasing concentrations of higenamine(●), non-radioactive dopamine(○), apomorphine(△), bromocriptine(▲), sulpiride(□) and metoclopramide(▽) were added to tubes containing 5 nM ^{3}H-dopamine and membrane preparation.

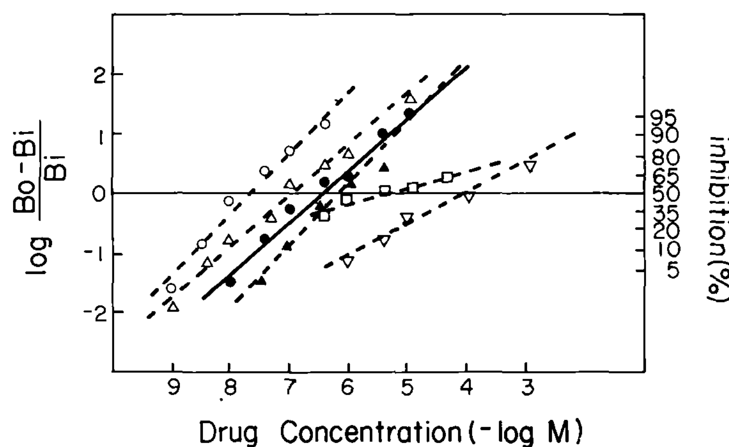

Fig. 7. Logit-log inhibition plot of ^{3}H-dopamine binding to D-2 receptor in the absence and presence of drugs. Data are from the same experiment as Fig. 6.

DISCUSSION

Although the classification of dopamine receptors has many controversy, dopamine receptors designated as D-1 and D-2 have received wide acceptance(Spano *et al.* 1980). Based on the pharmacological criteria and the regulation of activity of an identified enzyme, adenylate cyclase, D-1 dopamine receptor mediate the stimulation of adenylate cyclase activity while D-2 dopamine receptor is not associated with this enzyme activity(Kebabian and Calne 1979). Pharmacologically, dopamine, apomorphine and dopaminergic ergots such as bromocriptine are high affinity(nanomolar concentration) agonists to D-2 receptor and low affinity(micromolar concentration) agonist, antagonist or dualist to D-1 receptor while sulpiride is a relatively selective antagonist for D-2 receptor(Burt *et al.* 1975).

In the present study, ^{3}H-dopamine showed saturable specific binding to D-1 and D-2 receptors in bovine brain caudate nucleus membrane preparations disclosing Kd of 8.1 μM and Bmax of 116.5 pmol/mg protein at D-1 receptor and Kd of 7.9 nM and Bmax of 374.4 fmol/mg protein at D-2 receptor. The binding properties of higenamine on dopamine receptors are quite similar to those of known dopamine receptor agonists such as apomorphine and bromocriptine with respect to their Ki values.

Burt *et al.*(1976) found that the dopamine agonist has much higher affinity for ^{3}H-dopamine than for ^{3}H-haloperidol binding sites, and also found that the Hill coefficient for dopamine inhibition is 1.07, while for haloperidol inhibition of ^{3}H-dopamine binding the Hill coefficient is 0.51. From these findings, it has been claimed that there might be distinct dopamine receptor sites binding agonists and antagonists. In the present study, the Hill coefficient for bigenamine inhibition of ^{3}H-dopamine binding was 0.91 while those for sulpiride and metoclopramide inhibition were 0.22 and 0.59, respectively.

These and other results reported previously suggest that higenamine would have agonistic activity on dopamine receptors with higher affinity for D-2 receptor than for D-1 receptor.

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=국문초록=

Higenamine의 Dopamine수용체에 대한 결합특성에 관한 연구

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Higenamine의 Dopamine수용체에 대한 결합특성을 ^{3}H-dopamine 결합억제 방법을 이용하여 소의 뇌 caudate핵 세포막에서 관찰하였다.

^{3}H-dopamine의 D-2수용체에 대한 결합은 낮은 농도(nano-mole범위)의 higenamine으로 억제되었으나 D-1수용체에 대한 결합은 높은농도(micro-mole범위)의 higenamine으로 억제되었다.

^{3}H-dopamine결합에 대한 higenamine의 억제에 있어 Hill계수는 0.91이었으며 sulpiride와 metoclopramide에서의 Hill계수는 각각 0.22와 0.59였다.

이상의 결과로 미루어 higenamine은 D-1 수용체보다 D-2수용체에 대하여 높은 친화성을 가진 agonist활성을 나타낼 것으로 생각된다.