Dopamine Receptor Stimulating Effects of Chanoclavine Analogues, Tricyclic Ergot Alkaloids, in the Brain

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Accepted August 21, 1987

Abstract—The abilities of tricyclic ergot alkaloids, chanoclavine-I and its analogues, and bromocriptine to stimulate dopamine receptors in the brain were investigated. Receptor binding of $^3$H-spiperone has shown that bromocriptine exhibits clear affinity for this compound. The order of displacement potencies was bromocriptine >> ergometrine, KSU-1415 > chanoclavine-I, KSU-1118, KSU-1791. In the striatum of mice treated with an amino acid decarboxylase inhibitor, $r$-butyrolactone-induced DOPA accumulation was markedly inhibited by bromocriptine and KSU-1415, but not inhibited by chanoclavine-I. In mice with unilateral striatal 6-hydroxydopamine lesions, bromocriptine and KSU-1415 produced a long-lasting contralateral rotation that was suppressed by prior treatment with (±)-sulpiride. These results suggest that a tricyclic ergot alkaloid of the chanoclavine type stimulates D-2 receptors in the brain.

Chanoclavine-I, \((4R-(4a, 5\beta(E)))-2\)-methyl-3-\((1,3,4,5\text{-tетрахидро}-4\text{-}(\text{метиламин})\text{-benz(дс)индол}-5\text{-yl})\text{-2-пентен-1-ол, a tricyclic ergot alkaloid which occurs in the sclerotia of Claviceps purpurea Tul., has been regarded as a precursor of the tetracyclic ergolines, agroclavine, elymoclavine and lysergic acid amide (1); and its pharmacological effects have not been reported to date. Hence, we investigated the effects of chanoclavine-I and newly synthesized tricyclic alkaloids of the clavine type on dopamine receptors in vitro and in vivo. In this paper, we report that 4,5-trans-5-\((2\text{-метил-1-пентен-1-ил})\text{-4-пропиламино-1,3,4,5-тетрахидробенз(дс)индол (KSU-1415)) stimulates dopamine receptors in the brain of experimental animals.

Materials and Methods

Male Wistar rats (ST substrain, Shizuoka Lab. Animal Center, Shizuoka) weighing 180–200 g and adult male albino mice (ddY strain, Shizuoka Lab. Animal Center) weighing between 23–28 g were used. They were housed under uniform humidity, temperature, feeding and lighting conditions (on at 07:00, off at 19:00).

Receptor binding: Rat striata were homogenized in 50 volumes of ice-cold buffer (50 mM Tris/HCl, pH 7.7), centrifuged at 50,000×g for 10 min and washed by centrifugation twice. The pellet was suspended at 10 mg original wet weight/ml in 150 volumes of ice-cold buffer (50 mM Tris/HCl, pH 7.4, 120 mM NaCl, 5 mM KCl, 1 mM CaCl$_2$ and 1 mM MgCl$_2$) (2). Binding was determined by incubating 1.0 ml of membrane suspension with 0.15 nM of $^3$H-spiperone (Amersham Japan, 19.0 Ci/mmol) at 37°C for 10 min. The sample from each tube was filtered under vacuum through Whatman GF/B filters and then washed with two 5 ml rinses of ice-cold buffer. The filters were counted in Univer-Gel scintillation liquid. Specific binding was defined as that displaced by 10 μM (±)-sulpiride, and it represented 85% of the total binding at 0.15 nM $^3$H-spiperone.

Determination of DOPA and catechol compounds: Animals were sacrificed by decapi-
tation at the same time of day (13:30–15:00) after administration of drug. The brain was rapidly removed and placed ventral side up on a cold plate kept on ice. The striatum was cut out from the mouse brain with a razor blade and then frozen in liquid nitrogen. The paired striata of the mouse were homogenized in 1 ml of 0.25 N perchloric acid, containing 0.2 μM cysteine and 40 ng of 3,4-dihydroxybenzylamine (as an internal standard), and centrifuged at 10,000×g for 10 min. Catecholamines, 3,4-dihydroxyphenylalanine (DOPA) and 3,4-dihydroxyphenylacetic acid (DOPAC), were extracted with activated alumina and assayed using high performance liquid chromatography with electrochemical detection (LC-4B, Bioanalytical Systems, Inc.) as reported previously (3, 4).

Measurement of rotational behavior: Under pentobarbital anesthesia, 6-hydroxydopamine (6-OHDA, 8 μg) dissolved in sterile saline containing ascorbic acid (0.2 mg/ml) was injected into the right striatum of the mouse in a volume of 4 μl over 2 min according to the stereotaxic method (5). The coordinates of the injection site were A: 4.0, L: 2.0, H: +2.5, according to the stereotaxic atlas of the mouse brain (6).

Rotational behavior was directly observed in the plastic cage with a flat floor, dimensions of 24×17×12 cm³, and a 360° turn was designated as one rotation. Apomorphine HCl (0.1 mg/kg) was given to mice 7 days after 6-OHDA treatment, and mice that exhibited 200–300 contralateral turns in total were used in the present experiment.

Drugs: Apomorphine hydrochloride (Sigma), 6-hydroxydopamine hydrobromide (Sigma), m-hydroxybenzylhydrazine dihydrochloride (NSD-1015, Sigma) and ergometrine maleate (Tokyo Kasei) were dissolved in the sterile saline containing ascorbic acid (0.2 mg/ml). Bromocriptine mesylate (Sandoz), (±) and (−)-sulpiride (Fujisawa), and KSU-1415 were suspended in sterile saline with gum arabic. 1-Butyrolactone (Tokyo Kasei) was suspended in sterile saline. Compounds of the chanoclavine type (Fig. 1) were synthesized as reported elsewhere (7, 8). The doses of drugs were expressed as the amount of the respective base except for apomorphine and bromocriptine.

Fig. 1. The structures of chanoclavine-I and its derivatives, and hypothetically, how they are related to tetracyclic alkaloids of the clavine-type.
Data analysis: Data were analysed according to one way analysis of variances with a multiple comparison test (9) or the Mann-Whitney U-test.

Results
Scatchard plot of the specific binding isotherm yielded an apparent dissociation constant (Kd) of 0.23 fmol and a receptor density of 350 fmol/mg protein. Specific binding of ³H-spiperone to membranes of the rat striatum was displaced by small concentrations of bromocriptine, a reference ergot alkaloid. KSU-1415 inhibited the specific binding at relatively greater concentrations (IC50=1.9 µM) than those of bromocriptine (IC50=5.6 nM). Chanoclavine-I and other tricyclic compounds caused an inhibition of the specific binding at concentrations of over 10,000. IC50 values for the specific binding of 0.15 nM ³H-spiperone to striatal membranes are shown in Table 1.

To test whether tricyclic ergots stimulate presynaptic dopamine receptors in the striatum, the effects on DOPA accumulation in the mouse striatum treated with the amino acid decarboxylase inhibitor NSD-1015 plus γ-butyrolactone were measured. γ-Butyrolactone has been shown to elevate in vivo tyrosine hydroxylase activity in the striatum due to the cessation of nerve impulse flow in nigro-striatal dopaminergic neurons (10). The 9-ergolene type of ergot alkaloids ergometrine (10–40 mg/kg, i.p.) and bromocriptine (2.5–20 mg/kg, i.p.) inhibited γ-butyrolactone-induced DOPA accumulation in a dose-dependent manner. KSU-1415 (5–20 mg/kg) also inhibited DOPA accumulation induced by γ-butyrolactone dose-dependently, although other tricyclic ergots did not inhibit the accumulation at doses up to 50 mg/kg, i.p. IC50 values for striatal DOPA accumulation induced by γ-butyrolactone are given in Table 2. To investigate whether ergot alkaloids inhibit tyrosine hydroxylase through presynaptic dopamine receptors, effects of dopamine receptor antagonists on the alkaloid-induced inhibition of tyrosine hydroxylase were examined.

### Table 1. Inhibition of ³H-spiperone binding to rat striatal membranes

| Alkaloids   | IC50     |
|------------|----------|
| Bromocriptine | 5.6 nM   |
| Ergometrine  | 1.5 µM   |
| KSU-1415     | 1.9      |
| Chanoclavine-I | >10      |
| KSU-1118     | >10      |
| KSU-1791     | >10      |

Concentration required to inhibit the binding of 0.15 nM ³H-spiperone by 50% were determined from semi-logarithmic plots of concentrations of compounds against percent inhibition. The values are the averages of three to four separate experiments. * cited from ref. 18.

### Table 2. Inhibition of DOPA accumulation induced by γ-butyrolactone in the mouse striatum treated with m-hydroxybenzylhydrazine

| Alkaloids   | IC50 (mg/kg, i.p.) |
|------------|-------------------|
| Bromocriptine | 5.2               |
| KSU-1415     | 9.4               |
| Ergometrine  | 32                |
| KSU-1118     | >40               |
| KSU-1791     | >40               |
| Chanoclavine-I | no effect        |

Mice were treated with alkaloids for 30 min prior to the administration of γ-butyrolactone (750 mg/kg, i.p.), given m-hydroxybenzylhydrazine (100 mg/kg, i.p.) 5 min later, and sacrificed 30 min after the administration of m-hydroxybenzylhydrazine. IC50 values were determined graphically from the plots of concentrations of alkaloids against DOPA accumulation (n=4–5).
inhibition of DOPA accumulation were examined following the treatment with NSD-1015 alone. The inhibitory effects of KSU-1415 and bromocriptine on DOPA accumulation were significantly reversed by haloperidol (1 mg/kg, i.p.) and (-)-sulpiride (20 mg/kg, i.p.), respectively (Fig. 2).

The administration of KSU-1415 (5 and 10 mg/kg) slightly produced rotation contralateral to the side of the lesion. The administration of 20 mg/kg of KSU-1415 produced a marked increase in contralateral rotation, which persisted over 3 hr (Fig. 3). KSU-1118 (50 mg/kg, i.p.) produced a moderate rotation followed by long-lasting clonic convulsions. KSU-1791 (50 mg/kg, i.p.) caused a slight induction of rotation. Chanoclavine-I did not produce rotational behavior up to 50 mg/kg, i.p., but caused an induction of twitch responses on the back. Bromocriptine at a dose of 10 mg/kg, i.p., produced marked contralateral rotation over 3 hr, but only slight rotation at 5 mg/kg, i.p. Ergometrine (10 and 20 mg/kg, i.p.) also caused an increase in contralateral rotation. Rotational behavior induced by a high dose of KSU-1415 or bromocriptine was prevented by pretreatment with (±)-sulpiride (at a dose of 100 mg/kg) 1 hr prior to the alkaloids. Since the chanoclavine-type compounds have 4,5-trans and 4,5-cis stereoisomers, in vitro receptor binding and in vivo dopaminergic stimulating activities of both compounds were compared with each other, and the results indicated that the 4,5-trans compound was more active than the 4,5-cis one (data not shown).

Discussion

Agroclavine and elymoclavine, tetracyclic ergot alkaloids of the clavine type, have been described to have no significant pharmacological effects (11), and it is yet unclear whether these compounds have a direct metabolic relationship with chanoclavine-I (1). Hence, bromocriptine and ergometrine, 9-ergolene type of tetracyclic ergots, were used as reference drugs in the present study that confirmed the ability of both alkaloids to stimulate dopamine D-2 receptors in the brain. Chanoclavine-I showed a weak affinity for D-2 receptors in vitro, while it produced neither inhibition of striatal DOPA accumulation nor rotational behavior. These results

Fig. 2. Inhibitory effects of ergot alkaloids on striatal DOPA accumulation after decarboxylase inhibition. Mice were treated with KSU-1415 (20 mg/kg, i.p.) or bromocriptine (10 mg/kg, i.p.) for 30 min prior to NSD-1015 (100 mg/kg, i.p.) and sacrificed 30 min later. Haloperidol (1 mg/kg, i.p.) or (-)-sulpiride (20 mg/kg, i.p.) was given to animals 1 hr before the ergot alkaloids. The results expressed as values of the mean±S.E.M. of 4–5 experiments. *P<0.01 vs. NSD-1015 control, †P<0.01 vs. KSU-1415 or bromocriptine alone (Duncan's multiple test).
Tricyclic Ergots and Dopamine Receptors

may be due to a metabolic inactivation or an inhibition of dopaminergic functions in vivo (not examined). KSU-1118, the compound with the most simple skeleton among the chanoclavine-type compounds, showed the weakest affinity for dopamine receptors in vitro and in vivo, while an introduction of a n-propyl group to the nitrogen attached to the 4-position of benz(cd)indole (KSU-1415) increased the affinity for dopamine receptors in vitro and the stimulating activity of pre- and postsynaptic dopamine receptors in vivo as compared with the respective activities of KSU-1118. These results correspond with previous work indicating that N-propyl substitution leads to the most active compounds in a series of ergotline derivatives (12–14).

KSU-1415 possesses dopaminergic stimulating activity similar to that of bromocriptine in vivo. The very low activity of KSU-1415, in contrast to bromocriptine, in receptor binding studies suggest a possible biotransformation of the compound into active metabolite(s). The in vivo DOPA accumulation studies show that KSU-1415 has a relatively potent stimulating effect on presynaptic dopamine receptors in the striatum. Furthermore, the postsynaptic dopamine receptor stimulating effect of this compound is supported by the ability to produce contralateral rotation in mice with 6-OHDA lesions of the unilateral striatum (5, 15). In this behavior model, KSU-1415 is nearly half as potent as bromocriptine.

The fact that the in vivo dopaminergic stimulating effects of KSU-1415 are antagonized by sulpiride suggests that the compound stimulates D-2 receptors as does bromocriptine. By replacing the remaining hydrogen with one more n-propyl group, we expected to increase the dopaminergic stimulating effects as found in other ergot alkaloids (12–14). However, contrary to our expectations, the compound had markedly reduced affinity for dopamine receptors both in vitro and in vivo. This may be due to steric interactions between the 2-methylpropanoyl group and the two propyl groups attached to 5th and 4th position of the C ring, respectively.

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Fig. 3. Time course of ergot alkaloid-induced rotation in mice with unilateral striatal 6-hydroxydopamine lesions. *P<0.01 vs. those of lower doses (U-test).
Our results show that chanoclavine-I derivatives may join tetracyclic ergot alkaloids and simplified ergoline derivatives (14, 16) as dopamine receptor agonists. Furthermore, the results prompt us to synthesize new derivatives which will be devoid of the adverse reactions of bromocriptine (17).

Acknowledgment: We are grateful to Dr. Yoshikazu Kurashina, Director of the Creative Products Research Laboratories, Kissei Pharmaceutical Co., Ltd. for his support during this study.

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