Effect of Brain Lesions on [^{3}H]Ohmefentanyl Binding Site Densities in the Rat Striatum and Substantia Nigra

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We have recently demonstrated that [^{3}H]ohmefentanyl, a non-peptidergic opioid ligand which was suggested to cross the blood brain barrier in contrast to other peptidergic opioid ligands, bound not only to μ opioid receptor sites but also to σ sites. In order to examine whether [^{3}H]ohmefentanyl can be used as a marker for μ sites, we investigated the effects of brain lesions on [^{3}H]ohmefentanyl binding site densities, as compared with [^{3}H][D-Ala^2, MePhe^4, Gly-ol^5]enkephalin ([^{3}H]DAGO), a selective μ ligand. These binding site densities were measured by quantitative autoradiography in the rat striatum and substantia nigra, two brain structures known to contain a high density of μ receptors, following lesions of the nigro-striatal dopaminergic pathway and striatal intrinsic neurons. Following unilateral nigral lesion with 6-hydroxydopamine, [^{3}H]ohmefentanyl binding site densities were decreased in the patches (−35%) and matrix (−20%) of the ipsilateral striatum and in the lesioned substantia nigra pars compacta (−49%). Unilateral striatal lesion with quinolinic acid induced 72%, 61% and 50% decreases in [^{3}H]ohmefentanyl binding in the patches and matrix of the lesioned striatum and in the ipsilateral substantia nigra pars reticulata, respectively. Similar results were obtained in the binding of [^{3}H]DAGO. Indeed, a significant linear correlation was observed between [^{3}H]-ohmefentanyl and [^{3}H]DAGO binding site densities. Therefore, μ opioid receptors may be mainly located on intrinsic neurons in the striatum, dopaminergic cell bodies in the substantia nigra pars compacta and nerve terminals of striatal efferents in the substantia nigra pars reticulata.

The present study revealed that [^{3}H]ohmefentanyl binding sites follow a pattern similar to that observed for μ opioid receptors in response to lesions of the nigro-striatal dopaminergic pathway and striatal intrinsic neurons. Possible binding of [^{3}H]ohmefentanyl to σ sties may not influence changes in μ sites caused by such lesions. [^{3}H]Ohmefentanyl may thus be a useful tool as a marker for μ opioid receptors.

Keywords [^{3}H]ohmefentanyl; [^{3}H][D-Ala^2, MePhe^4, Gly-ol^5]enkephalin ([^{3}H]DAGO); μ opioid receptor; autoradiography; brain lesion; 6-hydroxydopamine; quinolinic acid; striatum; substantia nigra; binding site

Introduction

Ohmefentanyl is a 3-methylfentanyl derivative with an analgesic activity 6300 times more potent than that of morphine in mice.[2] Interestingly, it has been suggested that ohmefentanyl can cross the blood brain barrier[2] in contrast to a number of other peptidergic opioid ligands. Recently, we applied [^{3}H]ohmefentanyl to *in vitro* autoradiography in the normal control rat[3] and human brain,[4] and revealed a distribution closely related to that of [^{3}H][D-Ala^2, MePhe^4, Gly-ol^5]enkephalin ([^{3}H]DAGO), a peptidergic ligand highly specific to μ opioid receptors.[5] However, we found that [^{3}H]ohmefentanyl could bind not only to μ but also to σ sites.[3]

It was demonstrated that μ opioid receptors were dense in the rat striatum and substantia nigra.[5-9] Lesion studies using 6-hydroxydopamine (6-OHDA) suggested that a significant portion of μ opioid receptors was located on dopaminergic nerve terminals in the striatum.[5,10-13] It was also reported that striatal lesions with exogenous excitotoxins such as kainic acid (KA) and ibotenic acid (IBO) resulted in a substantial reduction in μ opioid binding, suggesting that a certain proportion of these binding sites was also found on striatal intrinsic neurons.[5,10,13,14] In the substantia nigra, although electrophysiological studies[15] revealed different effects of morphine and naloxone on the substantia nigra pars compacta and reticulata, the subregional distribution of μ opioid receptors in this structure as well as modulation of these binding sites following selective brain lesions has not been well documented.

According to our previous study[3] showing that [^{3}H]-ohmefentanyl may also bind to σ sites, it may be possible that [^{3}H]ohmefentanyl and [^{3}H]DAGO binding sites are affected differently by lesions of the nigro-striatal dopaminergic pathway and intrastriatal or efferent striatal neuronal networks. In order to check this hypothesis, in the present study we investigated the effect of nigral lesion with 6-OHDA and striatal lesion with quinolinic acid (QA), an endogenous excitotoxin, on [^{3}H]ohmefentanyl and [^{3}H]DAGO binding site densities by means of quantitative autoradiography. Indeed, it was shown that intrastriatal injections of KA[16-19] or IBO[20,21] destroyed not only striatal intrinsic neurons but also dopaminergic nerve terminals in the striatum, and QA was reported to produce more selective axon-sparing lesion.[21,22] However, QA has not yet been used to study the effect of striatal lesion on opioid receptors. A comparison between [^{3}H]ohmefentanyl and [^{3}H]DAGO binding site densities was then investigated following these lesions in the striatum and substantia nigra separated into pars compacta and reticulata.[8]

Materials and Methods

Surgical Procedures Male Wistar rats (200—250 g body weight) were anaesthetized with ketamine (150—175 mg/kg, i.p.) and fixed on a stereotaxic apparatus with incisor bars −3.3 mm from the interaural line. Eight μg of 6-OHDA hydrobromide (Sigma) in 2 μl of ice-cold Ringer's solution containing L-ascorbic acid (0.2 mg/ml) were injected into the left substantia nigra: AP +3.0 mm from the interaural line, L 1.8 mm, H +2.1 mm, calculated according to the atlas of Paxinos and Watson.[23] Injection was performed *via* a 25-gauge stainless steel blunt-tipped syringe needle attached to a Hamilton microsyringe (10 μl) mounted on a syringe infusion pump over a period of 2 min. Two μl of the vehicle used for 6-OHDA were injected under the same conditions for sham controls. The needle was left in place for an additional 3 min and then slowly withdrawn. The animals were sacrificed 14 d following the operation. For

the striatal lesions, 300 nmol of QA (Sigma) dissolved in 1 μl of phosphate-buffered saline (PBS), pH 7.4 were injected into the left striatum: AP +9.2 mm, L 2.5 mm, H +5.0 mm. Injection was carried out as described above over a period of 5 min. For the sham operation, 1 μl of PBS was injected under identical conditions. The animals were sacrificed 10 d after the operation.

Tissue Preparation and Control of the Lesions After sacrifice, the brains were immediately removed, frozen with dry ice, and cut into 10- and 300-μm-thick serial sections on a cryostat microtome. The 10-μm-thick sections were taken on gelatin-coated galss slides, and used for autoradiography. The 300-μm-thick sections were dissected in a cold box for biochemical analysis of the amount of γ-aminobutyric acid (GABA). Samples were punched out from almost all parts of the anterior striatum (precommissural level) and from the substantia nigra, including the pars compacta and reticulata. The GABA content was measured by the method of Graham and Aprison,[24] and the protein content was estimated by the method of Bradford.[25] The GABA levels were expressed as nmol/mg protein. Some slide-mounted 10-μm-thick sections were processed for [^{3}H]dihydrotetrabenazine (TBZOH) binding as previously described[26] to check the integrity of the dopaminergic innervation.[27] Briefly, sections were incubated with 12 nM [^{3}H]TBZOH (15 Ci/mmol, CEA, France) in 0.3 M sucrose, 10 mM *N*-hydroxyethylpiperazine-*N'*-2-ethansulfonate (HEPES), pH 8.0 at room temperature for 40 min. Non-specific binding was determined in the presence of 5 μM unlabeled tetrabenazine. The sections were washed 2 × 3 min at 4 °C in 40 mM Tris–HCl buffer, pH 8.0, and dipped in distilled water. Some radiolabeled sections were wiped off from the slides with Schleicher and Schuell filters, and their radioactivity contents measured with 5 ml of Aqualyte in a 1215 Rackbeta (LKB) liquid scintillation counter, with a 50% counting efficiency.[28]

Binding of [^{3}H]Ohmefentanyl and [^{3}H]DAGO Binding of [^{3}H]-ohmefentanyl was performed on slide-mounted 10-μm-thick sections as described elsewhere.[3] Briefly, slices were incubated with 2 nM [^{3}H]-ohmefentanyl (76 Ci/mmol, CEA, France) in 50 mM Tris–HCl, pH 7.4 at room temperature for 60 min. Additional sections were incubated in the presence of 0.25 μM unlabeled ohmefentanyl for the determination of non-specific binding. After incubation, the sections were rinsed 2 × 5 min in ice-cold 40 mM Tris–HCl buffer, pH 7.4, and dipped in distilled water. Adjacent sections were used for [^{3}H]DAGO binding. For that purpose, they were incubated for 60 min at room temperature with 3.4 nM [^{3}H]-DAGO (24 Ci/mmol, CEA, France) in 50 mM Tris–HCl, pH 7.4. Non-specific binding was performed in the presence of 0.25 μM unlabeled DAGO.[13] The sections were washed under the same conditions as described for [^{3}H]ohmefentanyl binding.

In these experiments, some radiolabeled sections were also wiped off from the slides with Schleicher and Schuell filters, and their radioactivity contents measured in a beta counter.[28]

Autoradiography Radiolabeled sections were dried under a stream of cold air. Film autoradiographs were obtained by the apposition of radiolabeled sections to Hyperfilm-^{3}H (Amersham) for 8 weeks for [^{3}H]-TBZOH, 10 weeks for [^{3}H]ohmefentanyl and 12 weeks for [^{3}H]DAGO, respectively, at room temperature in the dark. The films were developed and densitometric measurements were performed using an image analyzer (Biocom RAG200, Les Ulis, France), and converted into fmol/mg protein. The density of non-specific binding was subtracted from that of total binding.

Statistical Analysis Statistical analyses were carried out by means of Student's *t*-test in order to compare values in the region ipsilateral to the lesion and those in the corresponding structure on the same side of sham-operated animals. Effects of brain lesions on [^{3}H]ohmefentanyl and [^{3}H]DAGO binding site densities were compared in the regions ipsilateral to the lesions by linear regression. According to the two-tailed test, $p < 0.05$ was considered as a minimal level of significance.

Results

Control of the Lesions No change was observed in the GABA levels in both the striatum and substantia nigra following unilateral nigral lesion with 6-OHDA. In contrast, unilateral striatal lesion with QA caused a significant decrease in the GABA levels in both the ipsilateral striatum (7.8 ± 0.8 *vs.* controls 13.0 ± 1.0 nmol/mg protain (−41%)) and substantia nigra (79.0 ± 6.4 *vs.* 102.5 ± 5.1 nmol/mg protein (−23%)).

Unilateral nigral lesion with 6-OHDA caused significant decreases in [^{3}H]TBZOH binding site densities, a marker of the dopaminergic innervation[27] in the ipsilateral striatum (195 ± 19 *vs.* 796 ± 15 fmol/mg protein (−76%)) and substantia nigra pars compacta (55 ± 5 *vs.* 236 ± 10 fmol/mg protein (−77%)). No change was found in the ipsilateral substantia nigra pars reticulata, ventral tegmental area, nucleus accumbens and olfactory tubercle. Similarly, no change was found in the contralateral structures. Unilateral striatal lesion with QA induced no change in [^{3}H]TBZOH binding site densities in any of these structures.

[^{3}H]Ohmefentanyl Binding Measurement of radioactivity contents of whole tissue sections at the level of the striatum indicated that more than 90% of the total [^{3}H]ohmefentanyl binding was specific. As shown in Fig.

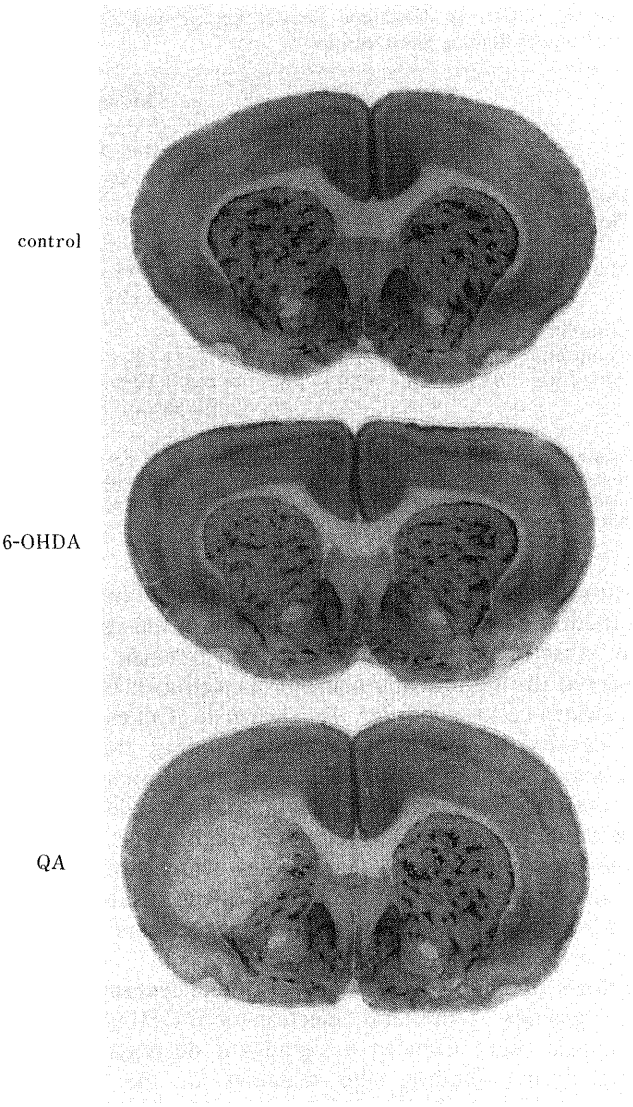

Fig. 1. Autoradiograms of [^{3}H]Ohmefentanyl Binding Sites on the Rat Brain Frontal Sections at the Level of the Striatum

Control: sham-operated control for unilateral nigral lesion with 6-OHDA. 6-OHDA: 14 d following injection of 6-OHDA (8 μg) into the left substantia nigra. QA: 10 d following injection of QA (300 nmol) into the left striatum. The vehicle was injected under identical conditions for each sham control. [^{3}H]Ohmefentanyl binding site densities in the striatum of sham controls for QA-lesioned animals were similar to those of sham controls for 6-OHDA lesion (see Tables I, II).

TABLE I. Effects of Unilateral Nigral Lesion with 6-OHDA on [^{3}H]-Ohmefentanyl Binding Site Densities

	Sham control		6-OHDA-lesioned	
	Ipsilateral	Contralateral	Ipsilateral	Contralateral
Striatum				
Patches	313.8±6.1	304.0±7.4	204.3±8.4[a] (−35%)	279.2±14.9
Matrix	123.5±3.2	126.9±3.2	98.5±3.2[a] (−20%)	114.7± 6.3
Substantia nigra				
Compacta	144.8±5.5	141.1±6.7	73.5±9.8[a] (−49%)	129.2± 7.4
Reticulata	102.2±4.9	96.8±3.5	85.9±6.9	109.2± 4.8

Data are expressed as fmol/mg protein, and are means±S.E.M. ($n=8$). The density of non-specific binding (10% of the total binding) was subtracted. Experimental conditions were described in Fig. 1. *a)* $p<0.01$ *vs.* corresponding side of sham-operated controls (Student's *t*-test).

TABLE II. Effects of Unilateral Striatal Lesion with QA on [^{3}H]-Ohmefentanyl Binding Site Densities

	Sham control		QA-lesioned	
	Ipsilateral	Contralateral	Ipsilateral	Contralateral
Striatum				
Patches	274.8±18.9	281.5±18.1	76.1±5.3[a] (−72%)	282.3±10.3
Matrix	112.6± 5.9	108.8± 8.7	45.1±1.9[a] (−61%)	119.6± 6.4
Substantia nigra				
Compacta	127.2± 9.0	131.3± 7.9	112.7±5.6	115.6± 5.4
Reticulata	93.2± 8.4	82.9± 5.6	46.9±3.1[a] (−50%)	76.2± 7.7

Data are expressed as fmol/mg protein, and are means±S.E.M. ($n=8$). The density of non-specific binding (10% of the total binding) was subtracted. Experimental conditions were described in Fig. 1. *a)* $p<0.01$ *vs.* corresponding side of sham-operated controls (Student's *t*-test).

TABLE III. Effects of Unilateral Nigral Lesion with 6-OHDA on [^{3}H]-DAGO Binding Site Densities

	Sham control		6-OHDA-lesioned	
	Ipsilateral	Contralateral	Ipsilateral	Contralateral
Striatum				
Patches	305.8±11.5	292.0±18.2	177.9±5.1[a] (−42%)	289.0± 9.7
Matrix	106.6± 8.7	100.0± 8.4	69.1±1.8[a] (−35%)	121.8± 9.9
Substantia nigra				
Compacta	122.5± 4.3	124.4± 3.8	54.4±5.1[a] (−56%)	130.1±10.3
Reticulata	93.9± 5.2	90.5±11.5	102.6±5.5	100.6± 3.8

Data are expressed as fmol/mg protein, and are means±S.E.M. ($n=8$). The density of non-specific binding (10% of the total binding) was subtracted. Experimental conditions were described in Fig. 1. *a)* $p<0.01$ *vs.* corresponding side of sham-operated controls (Student's *t*-test).

TABLE IV. Effects of Unilateral Striatal Lesion with QA on [^{3}H]-DAGO Binding Site Densities

	Sham control		QA-lesioned	
	Ipsilateral	Contralateral	Ipsilateral	Contralateral
Striatum				
Patches	357.8±17.2	296.5±18.7	95.6±10.5[a] (−73%)	307.1±12.7
Matrix	78.5± 5.5	75.9± 4.3	19.6± 3.9[a] (−75%)	65.4± 4.0
Substantia nigra				
Compacta	136.6±11.3	138.8± 5.8	117.6±12.6	123.1± 3.7
Reticulata	108.2± 2.1	94.0± 4.3	48.1± 4.8[a] (−56%)	99.7± 4.5

Data are expressed as fmol/mg protein, and are means±S.E.M. ($n=8$). The density of non-specific binding (10% of the total binding) was subtracted. Experimental conditions were described in Fig. 1. *a)* $p<0.01$ *vs.* corresponding side of sham-operated controls (Student's *t*-test).

1, autoradiographs of [^{3}H]ohmefentanyl binding sites resulted in a heterogeneous distribution in the striatum. In that structure, dense small clusters, patches, could be observed throughout the neuropil as well as a band along the striato-callosal border. As shown in Tables I and II, the density of [^{3}H]ohmefentanyl binding sites in the patches of sham control was 3 times more intense than in the matrix. Both pars compacta and reticulata of the substantia nigra showed relatively high levels of [^{3}H]-ohmefentanyl binding site densities which were almost similar to those found in the matrix of the striatum. However, the densities seemed higher in the pars compacta than in the pars reticulata.

Effects of Nigral Lesion on [^{3}H]Ohmefentanyl Binding Site Densities Unilateral injection of 6-OHDA into the substantia nigra induced a significant decrease in [^{3}H]-ohmefentanyl binding site densities in the ipsilateral striatum (Fig. 1, Table I). In the striatal patches, the magnitude of decrease was somewhat higher (−35%) than that of the matrix (−20%). No change was found in the contralateral striatum either in the patches or in the matrix. In the substantia nigra, [^{3}H]ohmefentanyl binding site densities were significantly decreased in the ipsilateral pars compacta (−49%) but not in the pars reticulata. The contralateral substantia nigra was not affected.

Effects of Striatal Lesion on [^{3}H]Ohmefentanyl Binding Site Densities As shown in Fig. 1 and Table II, unilateral striatal lesion with QA produced a marked decrease in [^{3}H]ohmefentanyl binding site densities in the patches in the ipsilateral striatum (−72%). The densities also showed an important but smaller decrease (−61%) in the matrix. No change was found in the contralateral striatum. In the substantia nigra, contrasting with the effect of nigral lesion with 6-OHDA, the [^{3}H]ohmefentanyl binding site densities were decreased after striatal QA injection in the pars reticulata (−50%) but not in the pars compacta. No change was found in the contralateral substantia nigra.

[^{3}H]DAGO Binding Measurement of radioactivity contents of whole tissue sections at the striatal level revealed that more than 90% of the total binding of [^{3}H]DAGO was specific. The distribution of [^{3}H]DAGO binding sites showed a pattern similar to that of [^{3}H]ohmefentanyl binding sites. [^{3}H]DAGO binding site densities were higher in striatal patches than in the matrix or in the substantia nigra (Tables III and IV).

Effects of Nigral Lesion on [^{3}H]DAGO Binding Site Densities Unilateral nigral lesion with 6-OHDA induced

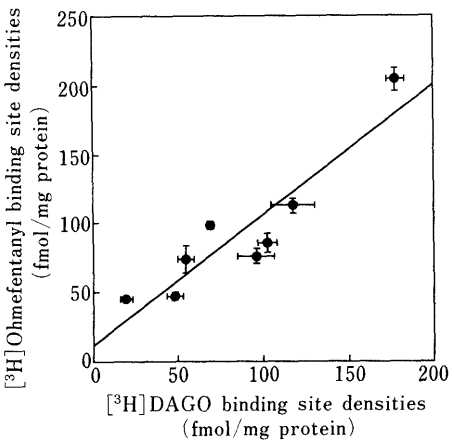

Fig. 2. Linear Correlation between [^{3}H]Ohmefentanyl and [^{3}H]DAGO Binding Site Densities in the Ipsilateral Striatum and Substantia Nigra Following Unilateral Nigral Lesion with 6-OHDA and Striatal Lesion with QA

The regression line was drawn by means of values in the brain structures ipsilateral to the lesions represented in Tables I—IV. $r=0.919$, $p<0.01$.

a significant decrease in [^{3}H]DAGO binding site densities in the ipsilateral striatum (Table III). In the patches, the magnitude of decrease was slightly higher than that in the matrix (patches: −42%, matrix: −35%). No change was found in the contralateral striatum. In the substantia nigra, [^{3}H]DAGO binding site densities were significantly decreased in the ipsilateral pars compacta (−56%) but not in the pars reticulata. The contralateral substantia nigra was not modified.

Effects of Striatal Lesion on [^{3}H]DAGO Binding Site Densities As shown in Table IV, unilateral striatal lesion with QA produced a marked decrease in [^{3}H]DAGO binding site densities in the ipsilateral striatum, both in patches (−73%) and matrix (−75%). No change was found in the contralateral striatum. In the ipsilateral substantia nigra, [^{3}H]DAGO binding site densities were decreased in the pars reticulata (−56%) but not in the pars compacta. Similarly, no change was found in the contralateral substantia nigra.

Comparison of Modifications in Binding Sites for [^{3}H]-Ohmefentanyl and [^{3}H]DAGO As shown in Fig. 2, a significant linear correlation was observed between [^{3}H]-ohmefentanyl and [^{3}H]DAGO binding site densities in the striatum and substantia nigra on the side ipsilateral to lesions with 6-OHDA and QA ($r=0.919$, $p<0.01$ by Student's t-test).

Discussion

Autoradiographic images of [^{3}H]ohmefentanyl binding sites revealed a heterogeneous distribution in the striatum, *i.e.*, patches and matrix, in agreement with previous studies using various ligands for μ opioid receptors.[5-9,13,14] The efficacy of the lesions was demonstrated by the fact that 6-OHDA lesion caused decreases in [^{3}H]TBZOH binding sites[26,27,29] and intrastriatal injection of QA produced a decrease in the GABA levels in the ipsilateral striatum and substantia nigra.[21,30,31] Unilateral nigral lesion with 6-OHDA caused significant decreases in the densities of [^{3}H]ohmefentanyl and [^{3}H]DAGO binding sites in the ipsilateral striatum, suggesting that a certain proportion of μ opioid receptors are located on dopaminergic nerve terminals in the latter structure. This finding is consistent with previous studies using [^{3}H]DAGO,[5,10,13] [^{3}H]-naloxone[11,18] and [^{3}H]Leu^5-enkephalin.[12] However, previous autoradiographic studies demonstrated that 6-OHDA lesion caused decreases in [^{3}H]DAGO binding site densities in striatal patches with a lack of significant decrease in the matrix.[5,10] In the present study, decreases were observed in both patches and matrix, suggesting that μ opioid receptors are located on dopaminergic terminals of the nigro-striatal pathway in both these two striatal compartments. Although we have no clear explanation for this discrepancy, it should be noted that our data corroborate those of Gerfen *et al.*[32] showing by a different approach that dopaminergic fibers from the substantia nigra project to both striatal patches and matrix in the rat.

On the other hand, degeneration of striatal intrinsic neurons by QA resulted in more severe decreases in [^{3}H]-ohmefentanyl and [^{3}H]DAGO binding site densities in the striatum. It suggests that a great proportion of μ opioid receptors is located on striatal intrinsic neurons. Our results following striatal QA lesion give a more accurate localization of μ receptors in the striatum than previous data,[5,10,13,14] since KA and IBO caused extrastriatal neuronal damage[17,33] and destroyed not only striatal intrinsic neurons but also dopaminergic nerve terminals in the striatum.[18,19,21]

In the present work we also described the effects of brain lesions on binding site densities for μ-specific ligand in the substantia nigra separated into pars compacta and reticulata. Unilateral nigral lesion with 6-OHDA resulted in decreases in [^{3}H]DAGO binding site densities in the lesioned substantia nigra pars compacta, suggesting that μ opioid receptors are located on dopaminergic cell bodies in that structure. Similar results were observed in [^{3}H]-ohmefentanyl binding sites. This finding extends a previous study,[12] performed with brain membranes, showing that [^{3}H]naloxone binding was decreased in the substantia nigra following nigral lesion with 6-OHDA. Recently, Waksman *et al.*[13] reported a lack of modification in [^{3}H]DAGO binding site densities in the substantia nigra after 6-OHDA injection into the medial forebrain bundle. However, these authors measured the binding site densities in the whole substantia nigra, while the decrease in the present study was only observed in the substantia nigra pars compacta following intranigral injection of 6-OHDA.

Furthermore, we observed here that striatal QA lesion induced significant decreases in the densities of [^{3}H]-ohmefentanyl and [^{3}H]DAGO binding sites in the substantia nigra pars reticulata. This finding suggests that μ opioid receptors are located presynaptically on axon terminals of long-projecting striatal neurons to the substantia nigra pars reticulata.[34,35] Taken together, μ opioid receptors may be located on dopaminergic and non-dopaminergic neurons in the substantia nigra pars compacta and reticulata, respectively. It is interesting to note here that previous electrophysiological studies[15] showed that systemic administration of morphine to the rat caused an increase in the firing rate of dopaminergic neurons in the pars compacta and a decrease in the activity of non-dopaminergic neurons in the reticulata, and these two kinds of alterations were reversed by naloxion. It may be possible

that opioid increases dopaminergic neurotransmission through both a direct activation in the substantia nigra pars compacta and an inhibition of non-dopaminergic neurons in the pars reticulata.

The present study demonstrated that [^{3}H]ohmefentanyl binding sites showed a localization quite similar to that of μ opioid receptors in the striatum and substantia nigra, as confirmed by [^{3}H]DAGO binding. Indeed, a significant linear correlation was observed between [^{3}H]ohmefentanyl and [^{3}H]DAGO binding site densities in these structures following both kinds of brain lesions (Fig. 2). Our recent studies[3,4] demonstrated that in rat and human brains [^{3}H]ohmefentanyl could in addition bind to a small proportion of σ sites. However, it does not seem that under our experimental conditions, [^{3}H]ohmefentanyl binding to σ sites is affected by such lesions. Although it was demonstrated that a small proportion of σ sites were present on dopaminergic cell bodies in the substantia nigra pars compacta and on dopaminergic terminals in the striatum,[36] possible alterations of [^{3}H]ohmefentanyl binding to σ sites may be masked by a major proportion of the binding to μ sites.

It has been suggested that ohmefentanyl, a new non-peptidergic opioid, can cross the blood brain barrier in contrast to the peptidergic compound, DAGO.[2] [^{3}H]-Ohmefentanyl may thus be a useful tool in further studies to determine μ opioid receptor distribution and regulation both *in vitro* and *in vivo*.

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