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Autoradiographic Mapping of Neurotransmitter System Receptors in Mammalian Brain

TSUTOMU ARAKI,*[1] HIROYUKI KATO,* KYUYA KOGURE,*
KATSURO SHUTO† AND YUKIHARU ISHIDA†

**Department of Neurology, Institute of Brain Diseases*
Tohoku University School of Medicine, Sendai, Japan
†Pharmacological Research Laboratory, Product Development Laboratories
Tokyo Tanabe Co. Ltd, Tokyo, Japan

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ARAKI, T., H. KATO, K. KOGURE, K. SHUTO AND Y. ISHIDA. *Autoradiographic mapping of neurotransmitter system receptors in mammalian brain.* PHARMACOL BIOCHEM BEHAV 41(3) 539–542, 1992.—Regional localization of neurotransmitter system receptors was visualized in the gerbil grain and in the rat brain using receptor autoradiography. [^{3}H]Quinuclidinyl benzilate (QNB), [^{3}H]cyclohexyladenosine (CHA), [^{3}H]muscimol, [^{3}H]MK-801, [^{3}H]SCH 23390, [^{3}H]PN200-110, [^{3}H]spiperone, and [^{3}H]naloxone were label muscarinic receptors, adenosine A_1 receptors, $GABA_A$ receptors, *N*-methyl-*D*-aspartate (NMDA) receptors, dopamine D_1 receptors, L-type calcium channels, spirodecanone receptors, and opioid receptors, respectively. Regional localization of [^{3}H]QNB, [^{3}H]CHA, [^{3}H]muscimol, [^{3}H]MK-801, [^{3}H]SCH 23390, and [^{3}H]PN200-110 binding sites in the gerbil brain was relatively similar to that in the rat brain. In contrast, the autoradiographic distribution of [^{3}H]spiperone and [^{3}H]naloxone binding sites in the gerbil was quite different from that in the rat. This phenomenon was found especially in the hippocampus and the cerebellum. The results suggest that the gerbil differs from the rat with respect to spirodecanone and opioid binding sites in the hippocampus and the cerebellum. This finding may help to further elucidate the species differences and relationships for brain function and behavioral pharmacology.

Receptor autoradiography Neurotransmitter system receptors Brain Gerbil Rat

IN the field of brain ischemia, gerbils and rats have been commonly used as experimental animals because they are relatively easy to produce and show good reproducibility (1,2, 10,19). On the other hand, gerbils are also used as a model of epilepsy because of the relative ease in eliciting seizures (13). A previous study suggested a loss of spines on pyramidal cell dendrites in the hippocampal CA3 sector of seizure-sensitive gerbils (17). Furthermore, Peterson et al. (18) demonstrated that seizure-sensitive gerbils showed a regional increase in the number of GABAergic neurons and terminals in dentate gyrus compared with seizure-resistant gerbils. These observations seem to suggest that the hippocampus plays an important role in the development of seizure activity. However, only a few investigations have been reported on major neurotransmitter binding sites in the hippocampus of the gerbil (14,15). Furthermore, little is known about the distribution of major neurotransmitter binding sites in the gerbil brain as compared with the rat brain under the same experimental conditions. In the present study, we focused on the major neurotransmitters and visualized the autoradiographic localization of these binding sites using receptor autoradiography in both gerbil and rat brain.

METHOD

Male Mongolian gerbils (n = 9, aged 4 weeks and weighing 30–36 g) and male Wistar rats (n = 7, aged 4 weeks and weighing 55–60 g) were used. Animals were killed between 1200 and 1300 by means of decapitation and brains were frozen in powdered dry ice. Saggital sections 12 μm in thickness were cut on a cryostat at −20°C and thaw mounted onto gelatin-coated cover slips. Sections were stored at −80°C until assay. Adjacent sections were stained with Cresyl violet and used for histopathology.

[1] Requests for reprints should be addressed to Tsutomu Araki, Ph.D., Pharmacological Research Laboratory, Product Development Laboratories, Tokyo Tanabe Co. Ltd, 33-3 Akabanekita 2-chome, Kita-ku, Tokyo 115, Japan.

[^{3}H]QNB Binding

Autoradiographic localization of muscarinic acetylcholine receptors was performed as reported previously (3). Sections were incubated with 1 nM [^{3}H]quinuclidinyl benzilate (QNB, 41.5 Ci/mmol, Amersham) in phosphate buffer (pH 7.4) for 90 min at room temperature. Sections were then washed in the buffer for 5 min at 4°C. Nonspecific binding was determined using 1 μM atropine.

[^{3}H]CHA Binding

The method for autoradiographic localization of adenosine A_1 receptors has been described previously (3). Sections were incubated with 5 nM [^{3}H]cyclohexyladenosine (CHA, 34.4 Ci/mmol, New England Nuclear) and 2 units/ml adenosine deaminase in 50 mM Tris-HCl buffer (pH 7.4) for 90 min at room temperature. Sections were then washed in the buffer for 1 min at 4°C. Nonspecific binding was determined using 10 μM L-phenylisopropyladenosine.

[^{3}H]Muscimol Binding

[^{3}H]Muscimol ($GABA_A$) autoradiography was done according to the method of Onodera et al. (14). Sections were subjected to 20-min prewash at 4°C in 50 mM Tris-citrate buffer (pH 7.1) to remove endogenous GABA and incubated for 40 min at 4°C in the buffer with 30 nM [^{3}H]muscimol (17.1 Ci/mmol, New England Nuclear). Sections were then washed in the buffer for 1 min at 4°C. Nonspecific binding was determined in the presence of 10 μM GABA.

[^{3}H]MK-801 Binding

Autoradiographic localization of [^{3}H]MK-801 binding was performed according to the method of Bowery et al. (5) with minor modifications. Sections were rinsed in 50 mM Tris-HCl buffer (pH 7.4) containing 190 mM sucrose and dried using a cold stream of air and incubated for 20 min at room temperature in the buffer with 30nM [^{3}H]MK-801 (28.8 Ci/mmol, New England Nuclear). Sections were then washed in the buffer twice for 20 s at room temperature. Nonspecific binding was determined used 100 μM MK-801.

[^{3}H]SCH 23390 Binding

Dopamine D_1 receptors were measured using [^{3}H]SCH 23390 [(*R*)-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1*H*-3-benzazepin-7-ol hemimaleate, a selective dopamine D_1 antagonist] by the method of Dawson et al. (7). Sections were incubated with 1 nM [^{3}H]SCH 23390 (87 Ci/mmol, New England Nuclear) in 50 mM Tris-HCl buffer (pH 7.4) containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, and 1 mM $MgCl_2$ for 30 min at room temperature. Sections were then dipped in the buffer at 4°C, followed by 25-min rinses in fresh buffer at 4°C. Nonspecific binding was determined using 10 μM SCH 23390.

[^{3}H]PN200-110 Binding

The method for the autoradiographic visualization of L-type calcium channel blocker binding using [^{3}H]PN200-110, a 1,4-dihydropyridine derivative, has been described previously (3). Sections were incubated with 0.1 nM [^{3}H]PN200-110 (71.5 Ci/mmol, New England Nuclear) in 170 mM Tris-HCl buffer (pH 7.7) for 60 min at room temperature. Sections were then washed in the buffer for 20 min at 4°C. Nonspecific binding was determined using 1 μM nitrendipine.

[^{3}H]Spiperone Binding

Autoradiographic localization of spirodecanone binding sites using [^{3}H]spiperone was visualized according to the method of Palacios et al. (16) with slight modifications. Sections were incubated with 1 nM [^{3}H]spiperone (23 Ci/mmol, New England Nuclear) in 170 mM Tris-HCl buffer (pH 7.4) for 40 min at room temperature. Also, various ions were included in the buffer: 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, and 1 mM $MgCl_2$. Sulpiride (10 μM) and mianserin (100 nM) were added to block dopamine D_2 and serotonin ($5\text{-}HT_2$) receptors, respectively. Sections were then washed in the buffer for 1 min at 4°C. Nonspecific binding was determined in the presence of 1 μM spiperone.

[^{3}H]Naloxone Binding

Opioid receptors were visualized using [^{3}H]naloxone according to the method of Herkenham and Pert (9) with minor modifications. Sections were incubated with 5 nM [^{3}H]naloxone (40 Ci/mmol, New England Nuclear) in 50 mM Tris-HCl buffer (pH 7.4) containing 100 mM NaCl for 60 min at 4°C. Sections were then washed in the buffer for 1 min at 4°C. Nonspecific binding was determined using 100 μM naloxone.

All procedures were performed under subdued lighting. Sections were dried under a stream of cold air and apposed to Hyperfilm-^{3}H (Amersham) for 2–8 weeks. In addition, nonspecific binding in the gerbil brain was similar to that in the rat brain.

RESULTS

Representative autoradiograms of [^{3}H]QNB, [^{3}H]CHA, [^{3}H]-muscimol, [^{3}H]MK-801, [^{3}H]SCH 23390, and [^{3}H]PN200-110 binding are shown in Fig. 1. In both gerbil and rat, the highest [^{3}H]QNB binding was noticed in the striatum, accumbens nucleus, and hippocampus, followed by the neocortex. Other regions had very low [^{3}H]QNB binding sites. [^{3}H]CHA binding was noticed in the neocortex, striatum, hippocampus, thalamus, substantia nigra, and cerebellum in both animals. In the rat, the superior colliculus also exhibited high [^{3}H]CHA binding sites, but the binding in this area was not noticed in the gerbil brain. Other regions also had relatively high [^{3}H]CHA binding sites. [^{3}H]Muscimol binding was relatively heterogenous and was highest in the neocortex, thalamus, and cerebellum, followed by the hippocampus, substantia nigra, striatum, and accumbens nucleus in both animals. Other regions exhibited low [^{3}H]muscimol binding sites. The highest [^{3}H]MK-801 binding was noticed in the hippocampus, followed by the neocortex, striatum, and thalamus in both animals. Other regions also exhibited relatively high [^{3}H]MK-801 binding sites except for the cerebellum and brainstem. Nonspecific binding was also seen under the present experimental condition, but it was homologous in both animals (data not shown). The results are consistent with a previous report (5). [^{3}H]SCH 23390 binding was strikingly heterogenous and was highest in the striatum, accumbens nucleus, and substantia nigra in both animals. The superior colliculus and dentate gyrus also exhibited high [^{3}H]SCH 23390 binding sites in the gerbil brain as compared with that in the rat brain. Other regions had extremely low [^{3}H]SCH 23390 binding sites except for the neocortex. [^{3}H]PN200-110 binding was noticed in the dentate gyrus and a part of the hypothalamus, followed by the neocortex, striatum, and thalamus in both animals. Other regions had low [^{3}H]PN200-110 binding sites.

Representative autoradiograms of [^{3}H]spiperone and

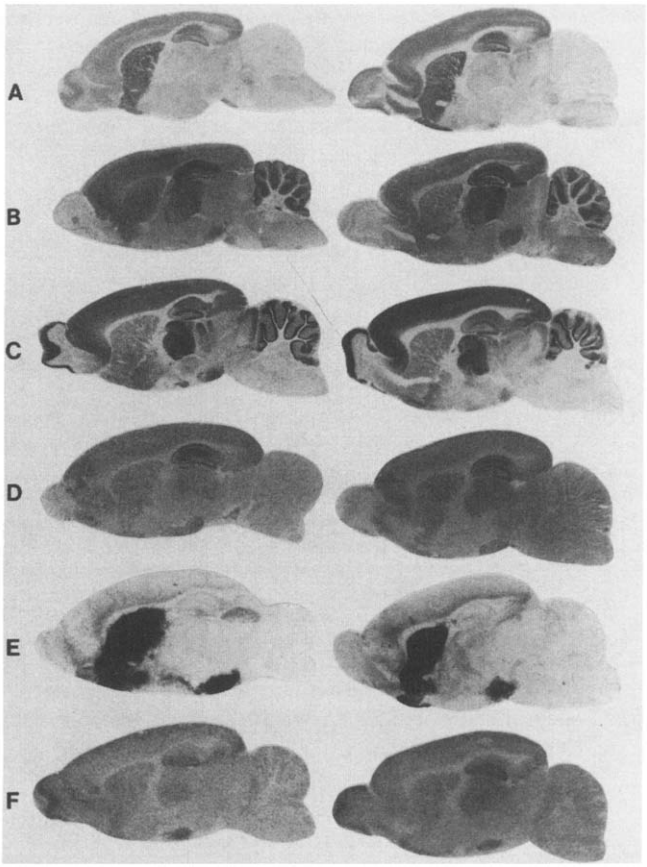

FIG. 1. Representative (A) [^{3}H]QNB, (B) [^{3}H]CHA, (C) [^{3}H]muscimol, (D) [^{3}H]MK-801, (E) [^{3}H]SCH 23390, and (F) [^{3}H]PN200-110 autoradiograms in gerbil (left) and rat brain (right). Regional localization of [^{3}H]QNB, [^{3}H]CHA, [^{3}H]muscimol, [^{3}H]MK-801, [^{3}H]SCH 23390, and [^{3}H]PN200-110 binding sites in gerbil brain was relatively similar to those in rat brain.

[^{3}H]naloxone binding sites and histological photographs are shown in Fig. 2. [^{3}H]Spiperone binding was found throughout the entire brain in both animals. However, the hippocampal CA1 sector exhibited very high [^{3}H]spiperone binding sites in the rat, but the binding was not noticed in the gerbil brain. [^{3}H]Naloxone binding was noticed in the striatum, accumbens nucleus, hippocampus, thalamus, hypothalamus, superior colliculus, and inferior colliculus in both animals. In the hippocampus, the gerbil had very low [^{3}H]naloxone binding activity in the CA1 pyramidal cell layer, but showed a high concentration of the receptors in the hilus and dentate gyrus. In contrast, the rat had extremely high [^{3}H]naloxone binding sites in the hippocampal CA1 pyramidal cell layer and hippocampal CA3 pyramidal cell layer, but the binding was not noticed in the hilus and dentate gyrus of the hippocampus. Furthermore, the cerebellum exhibited high [^{3}H]naloxone binding sites in the gerbil, but the binding activity was not noticed in the rat. The [^{3}H]naloxone binding in other regions of the gerbil brain was similar to that in the rat. For histological study, brains were intact in both animals.

DISCUSSION

The present study demonstrated that the regional localization of [^{3}H]QNB, [^{3}H]CHA, [^{3}H]muscimol, [^{3}H]MK-801,

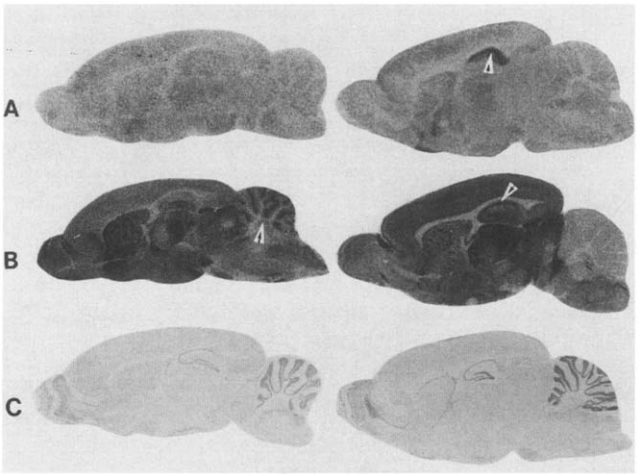

FIG. 2. Representative autoradiograms of (A) [^{3}H]spiperone and (B) [^{3}H]naloxone binding sites and (C) histological photographs in gerbil (left) and rat brain (right). Marked differences were noted in [^{3}H]spiperone binding sites in the hippocampal CA1 sector (arrow) and [^{3}H]naloxone binding sites in the hippocampal CA1 pyramidal cell layer (arrow) and the cerebellum (arrow). Furthermore, differences noticed in [^{3}H]naloxone binding in the hilus and dentate gyrus were relatively low in the rat but high in the gerbil. Brains were intact in both animals for histological study (Cresyl violet stain).

[^{3}H]SCH 23390, and [^{3}H]PN200-110 binding sites in gerbil brain was relatively similar to that in rat brain. However, a slight difference was seen in the superior colliculus in both [^{3}H]CHA and [^{3}H]SCH 23390 binding sites. In contrast, the autoradiographic distribution of [^{3}H]spiperone and [^{3}H]naloxone binding sites in the gerbil was quite different from that in the rat. This phenomenon was especially found in the hippocampus and cerebellum. Thus, the autoradiographic localization of these receptors in the gerbil brain differs from that in the rat brain.

It is of particular interest that marked differences were seen in the hippocampal CA1 sector, where opioid and spirodecanone binding sites were extremely low in the gerbil but high in the rat. The hippocampal CA1 sector is well known to play a key role in cognitive functions such as learning and memory (4,20). A previous study suggested that seizure-sensitive gerbils exhibit an increase in both the number of glutamic acid decarboxylase-positive somata and terminals in the dentate gyrus compared with seizure-resistant gerbils (18). Therefore, the hippocampus seems to play an important role in the development of seizure activity.

Pharmacological data provide strong evidence for different types of opioid receptors such as mu, delta, and kappa (6). In the present study, [^{3}H]naloxone predominantly labels mu receptors in the rat brain. However, [^{3}H]naloxone labels only mu receptors or other opioid receptors in the gerbil brain. Therefore, [^{3}H]naloxone binding sites in the present study remain to be determined. [^{3}H]Spiperone labels not only D_2 and 5-HT_2 receptors but also possibly another site on unknown significance, spirodecanone site. Our results are consistent with previous reports of a high density of spirodecanone binding sites in the rat hippocampal CA1 sector (11).

The present study indicated that the hippocampal CA1 sector exhibited very high [^{3}H]spiperone binding sites in the rat, but the binding was not seen in the gerbil brain. Furthermore, gerbils had very low [^{3}H]naloxone binding activity in the hip-

pocampal CA1 pyramidal cell layer, but showed a high binding activity of the receptors in the hilus and dentate gyrus. In contrast, rats had extremely high [^{3}H]naloxone binding sites in the hippocampal CA1 pyramidal cell layer and the hippocampal CA3 pyramidal cell layer, but showed very low [^{3}H]naloxone binding in the hilus and dentate gyrus. Thus, marked differences were seen in the hippocampus, where opioid and spiperone binding sites were extremely low in the gerbil but high in the rat. The high activity of [^{3}H]naloxone binding sites in the hilus and dentate gyrus may reflect an epileptic phenomena characteristic of gerbils. Interestingly, the cerebellum exhibited high [^{3}H]naloxone binding in the gerbil, whereas the binding activity was not noticed in the rat.

A previous study suggested that seizure-sensitive gerbils showed greater [^{3}H]naloxone binding, especially in the dentate gyrus, compared with seizure-resistant gerbils (12). Opioid peptides are known to cause excitation in the hippocampal pyramidal cell layer (8). From these observations, opioid peptide appears to be a key factor in modulating seizure activity and this peptide may be mediated by specific opioid binding sites. For [^{3}H]spiperone autoradiography, rats had high spirodecanone binding sites in the hippocampal CA1 sector, whereas gerbils had trace binding activity in this region. Interestingly, however, the cerebellum exhibited high [^{3}H]naloxone binding in the gerbil, but the binding activity was not noticed in the rat. Although the reason for the results is presently unclear, the findings are of interest in relation to mechanism of seizure. In addition, the regional distribution of [^{3}H]QNB, [^{3}H]CHA, [^{3}H]muscimol, [^{3}H]MK-801, [^{3}H]SCH 23390, and [^{3}H]PN200-110 binding sites in the gerbil brain was similar to that in the rat brain except for a few regions.

In conclusion, the present study suggests that the autoradiographic distribution of [^{3}H]naloxone and [^{3}H]spiperone binding sites in the gerbil is quite different from that in the rat. However, the regional localization of [^{3}H]QNB, [^{3}H]CHA, [^{3}H]muscimol, [^{3}H]MK-801, [^{3}H]SCH 23390, and [^{3}H]PN200-110 binding sites in the gerbil was relatively similar to that in the rat. The results indicate that the gerbil partly differs from the rat with respect to the hippocampus and the cerebellum. This finding may help to further elucidate the relationship or difference between gerbils and rats for brain function and behavioral pharmacology.

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