Quantitative Autoradiographic Localization of Prostaglandin E₂ Binding Sites in Monkey Diencephalon

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Quantitative autoradiography was performed to investigate the mapping of prostaglandin E₂ binding sites in the *Macaca fuscata fuscata* diencephalon. Autoradiographs were prepared by incubation of 10- μ m-thick serial frozen sections with ³H-prostaglandin E₂ and were processed by using a rotating drum-scanner and a computer-assisted image-processing system with ³H-microscales as standards. The localization of prostaglandin E₂ binding sites was remarkably discrete in the diencephalon. The highest concentrations were found in the median and medial preoptic areas, supra-mammillary nucleus of the hypothalamus, and centromedian nucleus of the thalamus. High density was observed in the medial and dorsal hypothalamic areas; paraventricular, anterior, dorsomedial, and infundibular nuclei of the hypothalamus; and in the anteroventral, periventricular, paraventricular, laterodorsal, and habenular nuclei of the thalamus. The distribution correlates well with the known effects of prostaglandin E₂ and may also give us useful clues in unveiling the novel role of prostaglandin E₂ in a variety of brain functions.

Prostaglandin (PG) E series has various neurophysiological functions, such as in the central mediation of fever (Milton and Wendlandt, 1970; Potts and East, 1972; Milton, 1976), as one of the major regulators of food intake (Scaramuzzi et al., 1970) and luteinizing hormone release (Ojeda et al., 1977; Kinoshita et al., 1982), and as an endogenous anticonvulsant (Poddubiuk, 1976; Rosenkrantz, 1978). The high-affinity and specific binding protein of ³H-PGE₂ was found in membrane preparations from bovine pineal gland (Cardinali et al., 1979) and from rat (Malet et al., 1982) and human (Watanabe et al., 1985a) brains. From a regional study of dissected brain areas, PGE₂ binding was found to be high in the hypothalamus, pituitary, and amygdala of rat brain (Malet et al., 1982) and in the amygdala, hypothalamus, and hippocampus of postmortem human brain (Watanabe et al., 1985a). The PGE₂ receptor protein was solubilized

in our laboratory in an active form from porcine brain using 3-[(3-cholamidopropyl) dimethylammonio]-1-propane sulfonate (Yumoto et al., 1986a). Furthermore, PGE₂ binding to the receptor is sensitive to guanine nucleotide (GTP), and, after solubilization, the receptor and GTP-regulatory protein were separated by gel filtration using high-performance liquid chromatography (HPLC) and were reconstituted (Yumoto et al., 1986b). These results provide evidence that PGE₂ in the brain exerts various neurophysiological functions via specific receptor binding, and that GTP is involved in its regulation. Recently, we demonstrated the localization of PGD₂ binding by using *in vitro* autoradiography coupled with a computerized image-analysis system (Watanabe et al., 1983; Yamashita et al., 1983). The specific PGD₂ binding was predominantly localized in the gray matter in the rat brain (Yamashita et al., 1983) and, more precisely, in specific neuronal cells, such as the Purkinje cell layer in the swine cerebellum (Watanabe et al., 1983). Preliminarily, we obtained the distinct localizations of PGD₂, PGE₂, and PGF_{2 α} bindings in the monkey brain (Watanabe et al., 1985b, 1986). In this study, we developed a digital subtraction method for the quantitation of autoradiographs and demonstrated by detailed mapping the concentration of PGE₂ binding sites in the monkey diencephalon.

Materials and Methods

Tissue preparation. *Macaca fuscata fuscata* (female, 6.5–8.0 kg) was anesthetized with Ketalar (ketamine-HCl) and perfused via the left ventricle with cold 10 mM sodium phosphate-buffered saline (pH 7.4) containing 5% sucrose. The brain was rapidly removed, dissected into 3–4 mm-thick blocks (ca. 2.0 $\times$ 2.5 cm), and frozen on dry ice. Frozen serial sections of 10 μ m thickness were cut in a cryostat at -14°C and mounted on gelatin-coated glass slides.

³H-PGE₂ binding. All procedures of ³H-PGE₂ binding assay were performed at 4°C as described by Yamashita et al. (1983) and Watanabe et al. (1983), with a slight modification. After preincubation in four 150 ml changes of 50 mM Tris-HCl (pH 7.4) containing 0.1 M NaCl (buffer A) for a total of 1 hr, the tissue sections were incubated with 150–200 μ l (the volume varied depending on the size of section) of 20 nM ³H-PGE₂ in buffer A for 30 min with gentle shaking (60 times/min, 4 cm reciprocal). The sections were then rinsed in 4 sequential short (15 sec each) dips in 50 ml of buffer A, quickly splashed with distilled water, and air-dried. Nonspecific binding was obtained with the consecutive sections by the addition of 100 μ M unlabeled PGE₂ to the incubation mixture.

The ionic condition and the concentration of ³H-PGE₂ for the incubation of sections were determined according to the results of the ³H-PGE₂ binding to the P₂ fraction of porcine cerebral cortex (Yumoto et al., 1986a). The K_d value of the ³H-PGE₂ binding to that fraction was a few nanomolar at 37°C . Since the PGE₂ binding sites showed a temperature-dependent rise in affinity (K_d for PGE₂ binding to the P₂ fraction prepared from monkey diencephalon and amygdala, 1.1 nM at 37°C and

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Figure 1. Typical standard curve for the tissue equivalent tritium content versus relative optical density of each layer of ³H-microscales. ●, High-level microscale; ○, low-level microscale. Calibration curve (dotted line) was generated from the log-log relationship of the tritium content with the corresponding relative optical density for each layer in a high-level ³H-microscale: $Y = aX^b$, where $a = 0.00189$ and $b = 1.84$ ($r^2 = 0.9987$), and for each layer in a low-level ³H-microscale: $Y = cX + d$, where $c = 0.0776$ and $d = -1.40$. The relative optical density of the film background (BG) was 18.1. The standard deviation of the average density of each layer of low and high ³H-microscales was very small; for the SDs of the lower 3 layers of high-level microscale (1.3, 4.9 and 7.8 nCi/mg tissue), those most frequently used for calibration were 0.13, 0.072, and 0.070% of the average, respectively, on the basis of data from 10 separate densitometry readings.

14 nM at 4°C; Y. Watanabe, N. Yumoto, and M. Hatanaka, unpublished observations), 20 nM ³H-PGE₂ might be close to the saturated level of all the binding sites.

The association of the specific ³H-PGE₂ binding to the section reached a plateau within 30 min at 4°C. For the dissociation of bound ³H-PGE₂ from incubated sections, we have no extensive time course, but 1 or 2 further 15 sec dips of the section into the buffer following the routine 4 dips resulted in little change in the total and nonspecific bindings.

Autoradiography. After being dried in a desiccator, the slides were tightly juxtaposed with tritium-sensitive films (Ultrafilm, LKB) and stored at 4°C for 4 weeks. ³H-Plastic standards (³H-microscales; Amersham) were included in each cassette. After exposure, the films were developed in Kodak D19 at 20°C for 5 min and then fixed for 5 min. The nuclei were identified by toluidine blue staining on the adjacent sections, with reference to the stereotaxic atlases of Emmers and Akert (1963) and Kusama and Mabuchi (1970).

Computer-assisted image-processing system. Films were analyzed using a computerized image-analysis system, including a rotating-drum scanning densitometer (Model 2605; Kimoto, Tokyo) and a minicomputer system (ECLIPSE S/120; Data General, Westboro, MA). The rotating-drum scanning densitometer was used to convert the photometric data from the autoradiographs into the digital values of 0 to 255 (relative O.D. value). The digitized data were arranged in parallel with the optical density between 0 and 1.5. Sampling pitches were 50 μm. The array of optical density readings was processed through a minicomputer. These data were converted to pseudocolor images on an image display (1024 × 1024 elements).

To produce quantitative images of the autoradiographs of ³H-PGE₂ binding, we transformed the original O.D. value of the pixels (pictures +

elements) to the binding value (BD value) that was linearly related to the quantity of ³H-PGE₂ bound in each pixel. This transformation was processed via the following steps: First, the mean O.D. value of each layer of ³H-microscales measured by the rotating-drum scanner was plotted against each tritium content. These data were expressed as a power function, $Y = aX^b$, where a and b were different depending on the exposure periods, the batches, and the developing conditions of the films. Then, according to this standard curve, the original O.D. value of each pixel in the image was converted to the BD value (fmol ³H-PGE₂/mg tissue). This linearization procedure was performed for each sheet of autoradiographic film with a set of ³H-plastic standards.

After the conversion of the O.D. value to the BD value, further processing via subtraction between the 2 images (total and nonspecific) was performed. To superimpose the image of nonspecific binding precisely on that of total binding, we selected several characteristic points in both images. Using their locations, we transferred the image of the nonspecific binding to the position of the image of the total binding by parallel and rotational transforms (affine transforms by the least-squares method). In affine transforms, the BD value of each pixel of the image was obtained from that of the corresponding pixels of the original image by linear interpolation. After all positions of the images were put into exactly the same position, subtraction among the BD values was performed to give the image of the specific binding. To determine the average concentration of ³H-PGE₂ bound in various nuclei, a cursor box was positioned over each nucleus in the image of the specific binding on the color display. The average BD value within the cursor box was calculated by the computer.

Materials. [5,6,8,11,12,14,15-³H (N)]PGE₂ (165 Ci/mmol) and Omnifluor were purchased from New England Nuclear (Boston, MA). Unlabeled PGE₂ was the generous gift of Ono Pharmaceutical Co. (Osaka). Autoradiographic ³H-microscales (RPA 501, batch 3, and RPA 507, batch 1) were obtained from Amersham.

Results

A typical standard curve for transformation of the O.D. value to the BD value is shown in Figure 1. Both high and low microscales consisted of 8 layers. Eight points of high microscale were described as a power function, while 8 points of low microscale were fitted for a linear function. The boundary points between low and high microscales were fitted for both functions. Therefore, we employed a straight line for the standard curve that was between 0 and 1.3 nCi/mg tissue of the BD value and used a power function for the curve above 1.3 nCi/mg. In all cases in this study, the goodness of fit of the standard curve with high microscale was ascertained; the relative coefficients (r^2) were greater than 0.998.

According to the above-mentioned standard curve, the ³H-PGE₂ content bound in each pixel was calculated for the total and nonspecific binding. An example of this quantitation procedure is shown in Figures 2 and 3. Figure 2, *A, B*, represents the original autoradiograms of total and nonspecific binding of ³H-PGE₂ in coronal sections of monkey brain containing the preoptic area. Figure 3, *A, B*, represents the corresponding pseudocolor-coded images. While the nonspecific binding (Fig. 3*B*) was low and rather homogeneously distributed, the total binding (Fig. 3*A*) was remarkably concentrated in the medial preoptic area. The image of the specific binding (Fig. 3*C*) was then processed by subtracting the binding values of the image of nonspecific binding from those of total binding. The image of the specific binding exhibited a pattern very similar to that of the total binding. The proportion of specific binding to the total binding was calculated to be more than 80% in the medial preoptic area. A similar presentation of the percentage of specific binding in the specific binding image is also possible; a typical result is shown in Figure 4. The BD value of each pixel in the image of the specific binding was divided by that of the corresponding pixel in the image of the total binding to produce the image of the percentage of specific ³H-PGE₂ binding (Fig. 4*A*).

A**B**

Figure 2. Original autoradiograms of $^3\text{H-PGE}_2$ binding in coronal sections of monkey brain containing the preoptic area. The tissue section (10 μm thick) was incubated with 20 nM $^3\text{H-PGE}_2$ in 50 mM Tris-HCl (pH 7.4) containing 0.1 M NaCl, as described in Materials and Methods. *A*, Total binding. *B*, Nonspecific binding, obtained with the consecutive section by adding 100 μM unlabeled PGE_2 to the incubation mixture.

Figure 4*B* shows the percentage of the specific binding value of each pixel along the horizontal straight line in Figure 4*A*. The highest specific binding in the centromedian nucleus reached 80% of the total binding.

The localization of $^3\text{H-PGE}_2$ binding was characteristic and discrete throughout the diencephalon (Fig. 5). The average $^3\text{H-PGE}_2$ concentrations in various nuclei were calculated and are summarized in Table 1. For example, the anteroventral nucleus of the thalamus is seen in Figure 5, *C–G*, and the centromedian nucleus of the thalamus in Figure 5, *H–J*. Their respective lengths were at least 1.5 and 1.9 mm in the sagittal direction, as judged from the section thickness (10 μm) and the number of sections. Since the mean value of $^3\text{H-PGE}_2$ binding in each nucleus varied anteriorly to posteriorly, we selected the highest values for presentation in Table 1.

The densities of $^3\text{H-PGE}_2$ binding sites were by far the highest (≥ 30 fmol/mg tissue) in the median and medial preoptic areas (Fig. 5, *A, B*), supramammillary nucleus of the hypothalamus (Fig. 5, *M, N*), and centromedian nucleus of the thalamus (Fig. 5, *H–J, P*).

In the limbic forebrain, a moderately high density (16 fmol/mg tissue) of PGE_2 binding sites was observed in the anterior commissural nucleus (Fig. 5, *A, B*), and a moderate concentration (11–13 fmol/mg tissue) was observed in the lateral septum and accumbens nucleus (Fig. 5*A*).

In the hypothalamus, high densities (17–21 fmol/mg tissue) of PGE_2 binding sites were observed in the paraventricular nucleus, anterior nucleus, dorsal and medial hypothalamic areas,

dorsomedial nucleus, and infundibular nucleus. Moderate densities (12–14 fmol/mg tissue) were found in the lateral hypothalamic area and mammillary nucleus (Fig. 5, *C–G, M, N*).

In the thalamus, PGE_2 binding sites were the most discretely located. In addition to a very high density of binding sites in the centromedian nucleus, the nuclei surrounding the third ventricle, such as the anteroventral (Fig. 5, *C–G, O*), periventricular (Fig. 5, *D, E, O*), paraventricular (Fig. 5, *H–J*), and laterodorsal (Fig. 5, *H–J, P*) nuclei showed high densities (16–22 fmol/mg tissue), and the reuniens nucleus (Fig. 5, *E, N*) showed moderate density (11 fmol/mg tissue). The habenular nucleus (Fig. 5, *K, P*) also possessed a high concentration of PGE_2 binding sites.

In the midbrain, the annular nucleus (Fig. 5*L*) and oculomotor nucleus (Fig. 5*P*) displayed high densities (16–17 fmol/mg tissue) of the binding sites.

We employed 2 monkey brains in the quantitative study and 4 other brains in the qualitative study. We observed little heterogeneity among the brain specimens in the pattern of localization of PGE_2 binding sites throughout the diencephalon.

Discussion

A number of reports have depicted the central action of PGE_2 mainly on the basis of pharmacological studies. However, there had been no reports concerning the precise localization of PGE_2 receptors in the CNS nor in the peripheral tissue before our recent studies. High-affinity PGE_2 binding was demonstrated by the glass filter assay using membrane preparations of bovine pineal gland (Cardinali et al., 1979) and that of rat (Malet et

Figure 3. Left, Pseudocolor-coded images of $^3\text{H-PGE}_2$ binding in coronal sections of monkey brain containing the preoptic area. The tissue section (10 μm thick) was incubated with 20 nM $^3\text{H-PGE}_2$ in 50 mM Tris-HCl (pH 7.4) containing 0.1 M NaCl, as described in Materials and Methods. Nonspecific binding was obtained with the consecutive section by adding 100 μM unlabeled PGE_2 to the incubation mixture. The image of specific $^3\text{H-PGE}_2$ binding was processed by subtracting the values of the binding in the corresponding pixels of the superimposed image of nonspecific binding from those of the image of total binding. Scales relate colors to fmol $^3\text{H-PGE}_2$ bound/mg tissue. *A*, Image of total $^3\text{H-PGE}_2$ binding. *B*, Image of nonspecific $^3\text{H-PGE}_2$ binding. *C*, Image of specific $^3\text{H-PGE}_2$ binding calculated by image subtraction. *caud*, Caudate nucleus; *put*, putamen; *sept*, septum; *med. preop*, medial preoptic area; *c.c.*, corpus callosum; *ant. com*, anterior commissure. Original autoradiograms are shown in Figure 2, *A*, *B*.

Figure 4. Right, Pseudocolor-coded image of $^3\text{H-PGE}_2$ binding in a coronal section of monkey diencephalon containing thalamus. Scales relate colors to the percentage of the specific binding to the total binding. *A*, Image of percentage of specific $^3\text{H-PGE}_2$ binding calculated by image division. *B*, Plot of line profile. Ordinate exhibits the percentage values calculated along the horizontal straight line drawn in *A*. The line crossed the centromedian nucleus, which showed the highest density of PGE_2 binding in the thalamus.

al., 1982), porcine (Yumoto et al., 1986a), and human brain (Watanabe et al., 1985a). In 1981, we started to investigate the binding sites of $^3\text{H-PGD}_2$ in the rat and porcine brain by using an *in vitro* autoradiography technique (Watanabe et al., 1983; Yamashita et al., 1983). Thereafter, preliminary studies using

the monkey brain revealed the distinct localization of PGD_2 , PGE_2 , and $\text{PGF}_{2\alpha}$ binding sites in the diencephalon and limbic system (Watanabe et al., 1985b, 1986).

This paper presents the first detailed report of the quantitative localization of PGE_2 binding sites in the brain. Various sites of

Figure 5. Representative images of specific $^3\text{H-PGE}_2$ binding distribution in monkey diencephalon. The tissue sections (10 μm thick) were incubated with 20 nM $^3\text{H-PGE}_2$ in 50 mM Tris-HCl (pH 7.4) containing 0.1 M NaCl, as described in Materials and Methods. Nonspecific binding was obtained with the consecutive section by adding 100 μM unlabeled PGE_2 to the incubation mixture. Images of specific $^3\text{H-PGE}_2$ binding were prepared by subtracting the values of the binding in the corresponding pixels of the superimposed image of nonspecific binding from those of the image of total binding. *A–L*, Coronal sections from one monkey, and *M–P*, those from another monkey, to cover almost all the diencephalic regions. *A*, *LS*, lateral septum; *Acc*, accumbens nucleus; *PM*, median preoptic area. *B*, *NST*, nucleus stria terminalis; *NCA*, anterior commissural nucleus; *APL*, lateral preoptic area; *APM*, medial preoptic area. *C*, *AV*, anteroventral nucleus; *AH*, anterior hypothalamic nucleus; *AMH*, medial hypothalamic area; *a.c.*, anterior commissure. *D*, *Pv*, paraventricular nucleus; *PH*, paraventricular nucleus; *ADH*, dorsal hypothalamic area; *AMH*, medial hypothalamic area. *E*, *Reu*, reuniens nucleus; *ADH*, dorsal hypothalamic area; *AMH*, medial hypothalamic area. *F*, *DMH*, dorsomedial nucleus; *VMH*, ventromedial nucleus. *G*, *DMH*, dorsomedial nucleus; *ALH*, lateral hypothalamic area; *IH*, infundibular nucleus; *HyN*, hypophysis. *H*, *LD*, laterodorsal nucleus; *Pa*, paraventricular nucleus. *I*, *CM*, centromedian nucleus. *J*, *SN*, substantia nigra; *IP*, interpeduncular nucleus. *K*, *Hb*, habenular nucleus. *L*, *EP*, epiphysis; *CG*, central gray; *Ann*, annular nucleus. *M*, *SMH*, supramammillary nucleus; *MM*, mammillary nucleus; *Hipp*, hippocampus. *N*, *Reu*, reuniens nucleus. *O*, *AV*, anteroventral nucleus; *Pv*, paraventricular nucleus. *P*, *LD*, laterodorsal nucleus; *Hb*, habenular nucleus; *CG*, central gray; *N_{III}*, oculomotor nucleus; *LG*, lateral geniculate nucleus; *MG*, medial geniculate nucleus.

Table 1. PGE₂ binding levels in monkey diencephalon

	Brain region	Binding (fmol/mg tissue)
Limbic forebrain	LS, lateral septum	13
	Acc, accumbens nu.	11
	NST, nu. stria terminalis	8.0
	NCA, anterior commissural nu.	16
Preoptic area	PM, median preoptic area	30
	APM, medial preoptic area	62
	APL, lateral preoptic area	9.5
Hypothalamus	SOH, supraoptic area	<3
	SCH, suprachiasmatic nu.	not identified
	PH, paraventricular nu.	17
	AH, anterior hypothalamic nu.	18
	ADH, dorsal hypothalamic area	19
	AMH, medial hypothalamic area	21
	ALH, lateral hypothalamic area	14
	DMH, dorsomedial nu.	20
	VMH, ventromedial nu.	6.6
	SMH, supramammillary nu.	41
	IH, infundibular nu.	19
	MM, mammillary nu.	12
	ME, median eminence	not identified
Thalamus	AV, anteroventral nu.	16
	Pv, periventricular nu.	22
	Pa, paraventricular nu.	17
	Reu, reuniens nu.	11
	VA, ventroanterior nu.	<3
	VL, ventrolateral nu.	<3
	VP, ventroposterior nu.	<3
	LD, laterodorsal nu.	22
	LP, lateroposterior nu.	<3
	MD, mediodorsal nu.	<3
	CM, centromedian nu.	49
	LG, lateral geniculate nu.	<3
	MG, medial geniculate nu.	<3
	Hb, habenular nu.	24
	Pu, pulvinar	<3
Midbrain	SN, substantia nigra	7.0
	CG, central gray	5.6
	RN, red nu.	<3
	IP, interpeduncular nu.	8.6
	Ann, annular nu.	16
	N _{III} , oculomotor nu.	17

Frozen 10- μ m-thick sections were labeled with 20 nM ³H-PGE₂, as described in Materials and Methods, and apposed against LKB Ultrofilm for 4 weeks to generate autoradiographs. Brain sections were selected at 300–500 μ m intervals for the demonstration of typical brain structures. The concentration of PGE₂ binding was calculated by grid sampling of the specific ³H-PGE₂ binding images, and the maximum values were taken from the concentrations in several sections of the same structure.

the central action of PGE₂ were unveiled on the basis of the results of the nucleus-level localization. Some are closely related to the known functions of PGE₂, as defined by pharmacological studies discussed below, and others may be useful clues in elucidating the novel physiological roles of PGE₂ in a variety of brain functions.

In the present study, the density of binding sites was calculated quantitatively in terms of fmol/mg of tissue. The standard curves

for the calibration of the tissue tritium concentration were obtained from Amersham's ³H-microscales. Since the standard curve in each film was strictly interpolated between 16 points of ³H-microscales, the reproducibility and reliability of the data were ascertained; further, comparisons between the independent experiments could be made in a quantitative manner. Rainbow et al. (1984) pointed out a serious problem of the autoradiography technique, that is, the difference in autoabsorption between gray and white matter due to the greater density of lipids in the white matter, especially in the case of tritium-labeled ligands. Kuhar and Unnerstall (1985) and Geary et al. (1985) also reported such a difference, and Geary et al. (1985) demonstrated that the O.D. value of the gray matter paste containing a certain tritium concentration was ca. 2-fold higher than that of the white matter paste containing the equivalent amount of radioactivity. In the present case, i.e., the study of the total and nonspecific binding of ³H-PGE₂, very little radioactivity was distributed in the white matter. Even if the radioactivity in the white matter were double the value obtained from the autoradiographic study, the pattern of localization of ³H-PGE₂ would not be significantly altered. Especially, in this study, we focused on the localization of PGE₂ binding sites in the gray matter regions (Table 1).

Since Milton and Wendlandt (1970) studied the febrile response following central administration of PGE₁, and Vane (1971) observed the inhibition of prostaglandin biosynthesis by antipyretic drugs, a number of studies have been done; PGE₂ was finally proposed to be an intermediary substance in the preoptic/anterior hypothalamic genesis of fever (Milton, 1976; Wolfe and Cocceani, 1979). However, there were no reports as to whether PGE₂ actually binds to the thermoregulatory neurons in the preoptic area. Recently, by use of the push–pull cannula technique, Cocceani et al. (1987) assessed the enhancement of actual release of PGE₂ at discrete preoptic/anterior hypothalamic sites by systemic injection of exogenous (endotoxin) or endogenous (interleukin-1) pyrogens. Our finding that the high density of PGE₂ binding sites exists in the median and medial preoptic areas offers strong evidence for the central mediator role of PGE₂ in the febrile response. Moreover, we ascertained that the localization of PGE₁ binding is identical to that of PGE₂ in the monkey brain (Y. Watanabe, unpublished observations).

PGE₂ has been postulated as being involved in the modification of algesia (Poddubiuk, 1976; Horiguchi et al., 1986). Horiguchi et al. (1986) described the biphasic effect of PGE₂ in regulating pain responses after its intracisternal administration to mice, and suggested that the site of PGE₂ action might be located in the lower portion of the CNS, i.e., brain stem and spinal cord. On the other hand, a high concentration of opiate receptors was found not only in the lower part of the CNS, but also in the centromedian and parafascicular nuclei of the thalamus in the monkey brain (Wamsley et al., 1982). The thalamus is a relay point for the fibers carrying protopathic pain. The present results show that PGE₂ binding is highly concentrated in the centromedian nucleus of the thalamus, although we could not distinguish the parafascicular nucleus from the centromedian nucleus. In addition, we observed the localization of PGE₂ binding in the central gray (midbrain periaqueductal gray matter) of monkey brain. These observations suggest a role of PGE₂ in the control of pain at multiple sites in the brain.

The neuroendocrine role of PGs is most evident in the hypothalamohypophysial pathway (Hedge, 1977; Behrman, 1979). Intraventricular injection of PGE₂ and PGD₂ caused stimulation

and suppression, respectively, of pulsatile luteinizing hormone release (Kinoshita et al., 1982). These responses were due to the reduction or enhancement of the release of luteinizing hormone-releasing hormone (LHRH) from the hypothalamic nuclei. In the rat brain, a high level of ^3H -PGD₂ binding was observed in the arcuate nucleus (Yamashita et al., 1983), one of the major sites of LHRH production in the rat brain. Ojeda et al. (1982) reported that PGE₂ stimulated the secretion of LHRH from incubated median eminence of rats and postulated that PGE₂ acts as a presynaptic mediator of norepinephrine via the α -adrenergic receptor. In 1985, Dray et al. found episodic fluctuations of the hypothalamic PGE₂ binding capacity during the rat estrus cycle, i.e., a biphasic rise of PGE₂ binding activity just before and after the LH surge. In the present study, a high density of ^3H -PGE₂ binding sites was observed in the infundibular nucleus, which is adjacent to the median eminence, and is one of the sites of production of anterior pituitary hormone-regulating hormone in primates (Nieuwenhuys et al., 1981).

PGs may also be a potent regulator of autonomic function (Poddubiuk, 1976). By the use of a microinjection technique, Feuerstein et al. (1982) determined the hypothalamic sites for cardiovascular stimulation by PGE₂ to be in the dorsomedial and posterior hypothalamic nuclei of rats; this corresponds well with our finding that the PGE₂ binding concentration was dense in the dorsomedial nucleus of the monkey hypothalamus (Fig. 5, F, G).

Concerning food intake, the issues of the effect of intrahypothalamic injection of PGE₂ and of effective sites are both controversial; both are seemingly species-specific (Wolfe and Cocceani, 1979). The present results indicate moderately high density of PGE₂ binding sites in the lateral hypothalamic area, the feeding center. However, we observed a negligible amount of PGE₂ binding in the ventromedial nucleus of the hypothalamus, the satiety center.

Although we can propose the above-mentioned functions for some of the PGE₂ binding sites localized by quantitative autoradiography, the significance of the binding sites in most other nuclei remains unknown. Especially the supramammillary nucleus of the hypothalamus and the habenular nucleus were rich in PGE₂ binding (Table 1), but the precise functions of these nuclei have not yet been clarified. Using RIA and gas chromatography-mass spectrometry, Ogorochi et al. (1984) investigated the occurrence of PGE₂ in various regions of postmortem human brain. They found the PGE₂ level to be high in the preoptic area, hypothalamus, amygdala, nucleus accumbens, pineal body, and pituitary, which were also rich in PGE₂ binding (Watanabe et al., 1985a). However, because of the limitation of the methodology, the PGE₂ contents at the nuclear level were not determined. If the hypothalamic PGE₂ receptor fluctuates with an infradian rhythm, as does the menstrual cycle as described by Dray et al. (1985), the present quantitative autoradiography technique may be a useful tool for the analysis of the alteration of PGE₂ receptor density and its functional role in a particular nucleus.

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