β_1 - and β_2 -Adrenergic ^{125}I -Pindolol Binding Sites in the Interpeduncular Nucleus of the Rat: Normal Distribution and the Effects of Deafferentation

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The plasticity of the β_1 - and β_2 -adrenergic receptor subtypes was examined in the interpeduncular nucleus (IPN) of the adult rat. The β -adrenergic receptor antagonist ^{125}I -pindolol (^{125}I -PIN) was used in conjunction with the selective subtype antagonists ICI 118,551 and ICI 89,406 to determine the subnuclear distribution of β_1 - and β_2 -adrenergic receptors in this nucleus and to correlate the receptor distribution with the distribution of both noradrenergic afferents from the locus coeruleus (LC) and non-noradrenergic afferents from the fasciculus retroflexus (FR). The density of these binding sites was examined following lesions that decreased (LC lesions) or increased (FR lesions) the density of the noradrenergic projection in the IPN.

Quantitative radioautography indicated that β_1 -labeled binding sites account for the larger percentage of binding sites in the IPN. The β_1 -binding sites are densest in the those subnuclei that receive a noradrenergic projection from the LC: the central, rostral, and intermediate subnuclei. β_1 -binding sites are also homogeneously distributed throughout the lateral subnuclei, where there is no detectable noradrenergic innervation.

β_2 -binding sites have a more restricted distribution. They are concentrated in the ventral half of the lateral subnuclei, where they account for 70% of total ^{125}I -PIN binding sites. β_2 -binding sites are also present along the ventral border of the IPN. Some of this labeling extends into the central and intermediate subnuclei.

Bilateral lesions of the LC, which selectively remove noradrenergic innervation to the IPN, result in an increase in the β_1 -binding sites. Bilateral lesions of the FR, which remove the major cholinergic and peptidergic input from the IPN, elicit an increase in noradrenergic projections and a decrease in β_1 -binding sites. β_1 -binding sites thus exhibit both up-regulation and down-regulation which is correlated with the density of the noradrenergic projection. Our results suggest, therefore, that the density of β_1 -binding sites is regulated by noradrenergic input.

β_2 -binding sites increase in density in response to both

the LC and FR lesions, suggesting that they are postsynaptic to both of these afferents. The distribution suggests that some of these binding sites may reflect binding to glial cells. The β_2 -binding sites may therefore be regulated by both noradrenergic and non-noradrenergic mechanisms.

The plasticity of β -adrenergic receptors is well established in the CNS. Both denervation supersensitivity, in response to decreased availability of norepinephrine (NE) (Sporn et al., 1976, 1977; Minnemann et al., 1979a; Sharma et al., 1979; U'Prichard et al., 1979, 1980; Reisine, 1981; Dooley et al., 1983; Sutin and Minnemann, 1985), and denervation subsensitivity, in response to increased availability of NE or β -agonists (Wolfe et al., 1978; Harden et al., 1979; Minnemann et al., 1979a; U'Prichard et al., 1979, 1980; Reisine, 1981; Sutin and Minnemann, 1985; Dooley and Bittiger, 1987), have been documented. The β -adrenergic receptors include 2 subtypes with distinct pharmacological (Lands et al., 1967; Minnemann et al., 1979b) and structural characteristics (Stiles et al., 1983; Strader et al., 1987). In the PNS, these 2 subtypes also have distinct tissue and organ distributions.

Recent studies suggest that there may also be a differential anatomical localization of these subtypes in the CNS. The β_1 -receptor subtype accounts for the majority of β -adrenergic receptors in most areas of the rat brain, but the β_2 -receptor subtype predominates in the cerebellum (Minnemann et al., 1979b; Palacios and Kuhar, 1982; Rainbow et al., 1984). Most of the adaptive changes that have been recorded for the β -adrenergic receptors, following perturbations of the CNS, can be attributed to changes in the β_1 -receptor subtype (Minnemann et al., 1979a; U'Prichard et al., 1979; Dooley and Bittiger, 1987). In fact, no alterations of β_2 -receptors have been seen in cortex (Minnemann et al., 1979a; Levin, 1982; Dooley and Bittiger, 1987), hippocampus (Dooley and Bittiger, 1987), or striatum (Minnemann et al., 1982) following alterations in noradrenergic input.

These pharmacological and biochemical studies suggest that different mechanisms mediate the response of these 2 receptor subtypes to altered innervation. It is known that these 2 receptors have different affinities for the catecholamines NE and epinephrine (Lands et al., 1967), and it has been suggested that different endogenous inputs regulate these subtypes (Minnemann et al., 1979a). Recent studies suggest more complex forms of regulation, e.g., heteroregulation (Tassin et al., 1982, 1987; Stockmeier et al., 1985; Scott and Crews, 1986) or coregulation (Stallcup and Patrick, 1980; Agnati et al., 1983). Finally, β_1 - and β_2 -subtypes may differ to the extent that they are pre- or

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postsynaptically located or in the extent of binding to neuronal and non-neuronal cells.

The interpeduncular nucleus (IPN) is a suitable nucleus in which to examine some of the properties of adrenergic receptors. Its afferents and subnuclear organization are well characterized (Hamill et al., 1984, 1986b; Hemmendinger and Moore, 1984; Artymyshyn and Murray, 1985; Groenewegen et al., 1986; Shibata et al., 1986; Battisti et al., 1987; Contestabile et al., 1987; Eckenrode et al., 1987). The IPN receives a noradrenergic projection exclusively from the locus coeruleus (LC) (Levitt and Moore, 1979; Battisti et al., 1987) via the central tegmental tract, which terminates in specific subnuclei (Hemmendinger and Moore, 1984; Battisti et al., 1987). The majority of non-noradrenergic afferents to the IPN are provided by the fasciculus retroflexus (FRs). The FRs contain cholinergic afferents that project to the same targets as the noradrenergic afferents and tachykinin-containing afferents that project to different subnuclei (Hemmendinger and Moore, 1984; Artymyshyn and Murray, 1985; Groenewegen et al., 1986; Battisti et al., 1987; Contestabile et al., 1987; Eckenrode et al., 1987; Murray et al., 1988). The IPN is also known to contain a substantial number of β -adrenergic receptors (Palacios and Kuhar, 1982; Rainbow et al., 1984), but the subnuclear distribution of the receptor subtypes and their relation to the noradrenergic afferents have not been described.

The normal distribution and density of the β -adrenergic receptors in the IPN can be labeled with the antagonist ¹²⁵I-pindolol (¹²⁵I-PIN) (Rainbow et al., 1984). The use of the selective antagonists ICI 89,406 and ICI 118,551 permitted the determination of the relative proportions of the β_1 - and β_2 -subtypes; using appropriate conditions, there is 97% occupancy of one subtype by one of these ICI drugs with less than 3% occupancy of the other (Rainbow et al., 1984). We could therefore correlate the subnuclear distribution and density of these 2 subtypes with the specific distribution of both noradrenergic and non-noradrenergic afferents in the IPN.

Lesions studies allowed us to examine the response of the β_1 - and β_2 -binding sites to different types of deafferentation. Bilateral 6-hydroxydopamine lesions of the LC result in a permanent and selective depletion of noradrenergic innervation to the IPN (Battisti et al., 1987) and therefore provided information regarding the plasticity of the receptor subtypes in response to noradrenergic denervation, as well as the location of the binding sites in relation to the noradrenergic afferents. In contrast, bilateral lesions of the FR remove substantial non-noradrenergic input from the IPN (Artymyshyn and Murray, 1985; Groenewegen et al., 1986; Eckenrode et al., 1987) and elicit increased innervation by LC afferents in their normal target subnuclei (Battisti et al., 1987). These lesions allowed us to examine the plasticity of the subtypes in response to an increased noradrenergic innervation.

Mismatches between anatomical localization of receptors and their respective afferents are common in the adult CNS (reviewed by Herkenham, 1987), and we expected that this might apply to the subnuclear distribution of β -adrenergic receptors in the IPN as well. In subnuclei normally devoid of noradrenergic afferents but containing β -adrenergic receptors, these lesions would provide information regarding the response of the receptor subtypes to removal of non-noradrenergic input.

Finally, indirect evidence has led to the suggestion that the β_2 -receptor is associated with non-neuronal structures, including glial cells and blood vessels (Minnemann et al., 1979a, 1982;

Nahorski et al., 1979; Dooley and Bittiger, 1987). The IPN is bordered ventrally by a dense glial limitans and also contains prominent rows of blood vessels that penetrate the intermediate subnuclei. The distribution of the β_1 - and β_2 -binding sites in relation to these areas and the response of these binding sites to the various lesions would provide more direct evidence regarding the anatomical localization of the β -adrenergic receptors and the mechanisms that may govern their respective regulation.

Materials and Methods

Adult female Sprague-Dawley rats, weighing 150–174 gm, were ordered in groups of 18 animals from Ace Animals. The animals were housed 3 per cage, under 12 hr alternating light/dark cycles, and permitted unlimited access to food and water. They were allowed several days to acclimate to their new environment before surgery. The group was then divided into subsets consisting of a control group and bilateral FR and bilateral LC lesion groups. In addition, several sham-operated animals were included. Surgery was performed during the morning hours. Animals were anesthetized with chloral hydrate anesthesia (0.035 gm/100 gm body weight, i.p.).

Three groups of animals were studied: a control group and acute (1 week postoperative) and chronic (6 or 8 weeks postoperative) lesion groups. Following appropriate survival times, the animals were decapitated, and their brains were rapidly removed, frozen on powdered dry ice, and divided into one block containing the IPN and another block containing the lesion site. The blocks were stored in containers at -70°C until sectioning. All animals were killed during the late morning hours to control for diurnal variations.

LC lesions ($n = 12$). LC lesions were made by stereotaxic injection of 6 μg 6-hydroxydopamine base (6-OHDA), dissolved in 3 μl of 0.9% NaCl solution containing 1 mg/ml of ascorbic acid, and adjusted to pH 5.5. Animals were pretreated with pargyline (50 mg/kg in 0.9% NaCl, i.p.) 15–30 min prior to the infusion of the neurotoxin. The animals received bilateral infusions of freshly prepared 6-OHDA via a 5 μl Hamilton syringe fitted with a 24 gauge needle at the coordinates 1.1 mm caudal to the interaural line, ± 1.2 mm lateral to the sagittal suture, and 2.2 mm dorsal to the interaural line, with the jaw bar set at -2.5 mm (atlas of König and Klippel, 1963).

FR lesions ($n = 12$). FR lesions were made using a radiofrequency generator (Radionics RFG-4) at the coordinates 3.5 mm rostral to the interaural line, ± 0.6 mm lateral to the sagittal suture, and 4.4 mm dorsal to the interaural line, with the jaw bar set at -2.5 mm (atlas of König and Klippel, 1963). The electrode tip temperature was raised to 60°C and left in place 1 min.

Verification of lesions. Lesion blocks were serially sectioned (20 μm) in a cryostat. Coronal sections were thaw-mounted onto slides, stained with cresyl violet, dehydrated, and coverslipped with mounting medium. FR lesions were verified histologically. LC lesions were assessed by the location of the needle tract and the loss of the majority of the LC neurons. Previous experiments, using biochemical measurements, demonstrate this lesion results in a 75% depletion of NE in the IPN (Battisti et al., 1987).

Radioligand binding. Blocks of brain containing the IPN, stored at -70°C , were allowed to equilibrate to -16°C in a cryostat for 20 min, mounted on a chuck, and serially sectioned in a coronal plane at 24 μm . Sections were thaw-mounted onto chrome alum gelatin coated slides, one section per slide, and every 9th section through the IPN was stained with cresyl violet for histological identification of the subnuclei. The remaining slides were distributed in sets of 4 adjacent sections, in a series of black plastic boxes so that each box would contain the same level of the IPN from several different animals. The boxes were kept at -16°C in the cryostat until they were filled. The boxes were then transferred to a dessicator and stored under vacuum at 4°C overnight. The following day they were transferred to a deep freeze at -70°C and stored until the day of the binding assay. The slides were allowed to come to room temperature for 20 min. The radioautography procedure described by Rainbow et al. (1984) was used to determine the distribution of total β -adrenergic receptor sites and of the β_1 - and β_2 -subtypes. Slides of adjacent sections were distributed in 1 of 4 plastic mailers. Each mailer contained an incubation solution of 4 ml Tris-saline buffer (20 mM Tris HCl/135 mM NaCl, pH 7.4) and 200 pM iodopindolol (¹²⁵I-PIN; New England Nuclear). This is a ligand saturating concentration as determined by Rainbow et al. (1984). Sections incubated in buffered ¹²⁵I-

PIN were used to determine the distribution of total ^{125}I -PIN binding sites. The addition of either ICI 89,406 (35 nM), a selective β_1 -antagonist or ICI 118,551 (50 nM), a selective β_2 -antagonist to the buffered ^{125}I -PIN was used to determine the distribution of the subtypes (Rainbow et al., 1984). Nonspecific binding was determined from sections co-incubated in the presence of the agonist isoproterenol (150 μM), added to the 4th mailer. The slides were incubated for 70 min at 23°C, followed by 3 washes, 20 min each, in 4°C Tris-saline buffer (pH 7.4). They were then rinsed quickly in cold water to remove buffer salts, dried under a stream of cool air, and then stored in a dessicator at 4°C overnight to remove any remaining moisture. Each assay consisted of a matched level of the IPN from all of the control and lesions groups for both an acute (1 week) and chronic (6 or 8 weeks) survival period. At the section thickness we used, each level was represented over 2 series of 4 adjacent sections per animal.

Preparation of radioautograms. The slides were placed in x-ray cassettes with each series of 4 alternate sections ordered as follows: total ^{125}I -PIN binding sites, β_1 -binding sites, β_2 -binding sites, and nonspecific binding sites. Slides of sections from normal animals and those from the various lesion groups were distributed among the cassettes so that no one cassette contained only one type of lesion group or time course. In addition, each cassette contained one slide of brain mash standards, sectioned at 24 μm , calibrated for μCi of ^{125}I /mg protein. Slides were tightly apposed to ^3H -Ultrafilm (LKB; Gaithersburg, MD) and allowed to expose at room temperature for 17 hr. The film was then processed in Kodak GBX developer (3 min) and fixative (6 min).

Quantification of radioautograms. A Quantimet image-analysis system (Cambridge Systems) was used to measure densities of ^{125}I -PIN binding sites in the subnuclei of the IPN from normal and lesioned animals. Following their exposure to Ultrafilm, the sections were stained with cresyl violet, and the subnuclei present in each section were identified. Outlines of the IPN were drawn from these sections, with reference boundaries included to aid in the later alignment of these maps with their corresponding radioautograms. The subnuclear boundary map was then aligned with the radioautographic image and optical densities measurements were taken within the entire area designated by specific subnuclear boundaries of the IPN. Density measurements were recorded within each subnucleus of the IPN present at the rostral-caudal levels measured. Optical density measurements recorded from sections incubated in the presence of isoproterenol defined nonspecific binding, and these measurements were subtracted from the corresponding total ^{125}I -PIN binding, β_1 - and β_2 -binding site measurements. The reported values therefore reflect specific binding. Optical density measurements were converted into femtomoles of radioligand bound per mg protein from the brain mash standards exposed to each film from which the subsequent IPN optical density measurements were recorded.

The images corresponding to nondisplaceable binding were barely detectable above the background of the film, and these values ranged from 2–12% of total binding.

Area measurements of IPN subnuclei. Using the Quantimet image-analysis system, outlines of the IPN and its subnuclei were drawn from cresyl violet stained sections. Mean areas of the subnuclei from control and experimental animals were calculated and compared using a Student's *t* test.

Figure 1. Diagram of transverse sections showing subnuclei of the IPN at 2 rostrocaudal levels. $\times 60$. Subnuclei: R, rostral; C, central; I, intermediate; L, lateral; D, dorsal. *a*, Rostral level, approximately $1/3$ through the nucleus. *b*, Caudal level, approximately $2/3$ through the nucleus.

Results

Normal distribution

Two levels of the IPN were examined: a rostral level that contained the rostral subnucleus and the most rostral portions of the central, intermediate, and lateral subnuclei; and a caudal level that contained the dorsal subnucleus and the main portions of the central, intermediate, and lateral subnuclei (Fig. 1).

Distribution of total ^{125}I -PIN binding sites in the IPN

^{125}I -PIN binding sites to β -adrenergic receptors are present in all of the subnuclei at the 2 levels examined, but their density and distribution among the subnuclei varies. At the rostral level, the densest concentration occurs as a narrow band along the entire ventral border of the IPN, which is composed primarily of a dense glia limitans. The relative density of total labeling is otherwise similar among the subnuclei. At the caudal level, a narrow band of dense labeling is also present along the ventral border of the IPN. This band of labeling widens and extends into the ventral half of the lateral subnuclei. This is the area of the densest ^{125}I -PIN binding sites within the IPN. The remaining portions of the lateral, intermediate, and central subnuclei are less densely labeled. The dorsal nucleus has the lowest concentration of ^{125}I -PIN binding sites (Table 1).

Distribution of the β_1 - and β_2 -subtypes

Inhibition of ^{125}I -PIN binding by the selective β_1 -receptor antagonist ICI 89,406 reveals the distribution of β_2 -adrenergic ^{125}I -PIN binding sites; for convenience, we will refer to this labeling as β_2 -binding sites. β_1 ^{125}I -PIN binding sites are revealed

Table 1. Mean density of ^{125}I -PIN binding sites in subnuclei of the interpeduncular nucleus of control rats (fmol binding/mg protein $\pm$ SE)

Subnuclei	Total binding	β_1 Binding	β_2 Binding	% β_1	% β_2
Lateral (rostral)	57.8 $\pm$ 3.0	28.3 $\pm$ 1.2	28.8 $\pm$ 1.4	50.0	49.8
Lateral (caudal)	65.9 $\pm$ 1.9	28.0 $\pm$ 1.4	37.1 $\pm$ 1.8	42.5	56.3
Intermediate	61.5 $\pm$ 1.4	35.8 $\pm$ 1.4	22.6 $\pm$ 1.9	58.2	36.7
Central	59.0 $\pm$ 2.1	37.7 $\pm$ 1.2	23.4 $\pm$ 1.5	63.9	39.7
Rostral	54.4 $\pm$ 2.3	34.2 $\pm$ 3.3	18.6 $\pm$ 1.0	62.9	34.2
Dorsal	40.8 $\pm$ 1.9	30.1 $\pm$ 1.2	12.2 $\pm$ 0.8	73.8	29.9

The table gives the density of total ^{125}I -PIN binding sites, β_1 -binding sites, and β_2 -binding sites in the subnuclei of the IPN from control rats ($n = 6$). Only the lateral subnuclei show a rostral-caudal difference in density of binding sites (unpaired *t* test: total ^{125}I -PIN, $p \leq 0.05$; β_2 -binding sites, $p \leq 0.05$). Unpaired *t* test of all other subnuclei that are present at both levels examined showed no significant difference in binding density. Therefore, values presented for the central and intermediate subnuclei represent pooled averages for each animal across the 2 levels.

Figure 2. Digitalized image of radioautogram of control IPN at a rostral level corresponding to level shown in Figure 1a. Adjacent sections through the IPN show total 125 I-PIN, β_1 - and β_2 -binding sites. Drawings made from cresyl violet stained sections were aligned over the radioautographic image of the same sections to determine boundaries of the IPN and to identify the subnuclei. Dashed line in the first section of a series outlines the IPN. Subnuclei: R, rostral; C, central; I, intermediate; L, lateral. Scale (to left of figures) represents optical density values. $\times 60$. *a*, Distribution of total 125 I-PIN binding sites. *b*, β_1 -Binding sites are present in all subnuclei of the IPN but are more concentrated in those subnuclei that receive noradrenergic innervation from the LC. *c*, β_2 -Binding sites are concentrated along the ventral border of the IPN.

using the β_2 -selective receptor antagonist ICI 118,551, and we will refer to this labeling as β_1 -binding sites. The use of these selective receptor antagonists shows that the β_1 - and β_2 -binding sites have markedly different distributions among the subnuclei of the IPN.

The β_1 -binding site distribution most closely correlates with the distribution of noradrenergic afferents to the IPN as described previously, i.e., the central, intermediate, and rostral subnuclei (Hemmendinger and Moore, 1984; Battisti et al., 1987). β_1 -Binding sites are homogeneously distributed among these

Figure 3. Digitalized images of radioautograms of the IPN from control and experimental animals at a caudal level corresponding to level shown in Figure 1*b*. Drawings made from cresyl violet stained sections were aligned over the radioautographic image of the same sections to determine boundaries of the IPN and to identify the subnuclei. *Dashed line* in the first section of a series outlines the IPN. Subnuclei: C, central; I, intermediate; L, lateral; D, dorsal. *Scale* (to left of figures) represents optical density values. Identical scales are used across animal groups. $\times 30$. *a-c*, Adjacent sections of the IPN from a control animal. *a*, Distribution of total ^{125}I -PIN binding sites in control IPN. *b*, β_1 -Binding sites are distributed through all the subnuclei at this level. *c*, β_2 -Binding sites account for area of densest labeling in the IPN and are concentrated in ventral half of lateral subnuclei at this level. This area receives no detectable noradrenergic innervation. *d-f*, Adjacent sections of the IPN from an animal with a chronic bilateral LC lesion that eliminated noradrenergic input to the IPN. *d*, Distribution of total ^{125}I -PIN binding sites. *e*, β_1 -Binding sites increase in density in response to this lesion. *f*, β_2 -Binding sites also increase in density in the subnuclei they normally occupy. *g-i*, Adjacent sections of the IPN from an animal with a chronic bilateral FR lesion that increased noradrenergic innervation to the IPN and removed substantial non-noradrenergic input. *g*, Distribution of total ^{125}I -PIN binding sites. *h*, β_1 -Binding sites decrease in density in most of the subnuclei they normally occupy. *i*, β_2 -Binding sites increase in density in response to this lesion.

subnuclei. β_1 -Binding sites are also found, to a lesser extent, in the lateral subnuclei and in the dorsal subnucleus (Figs. 2*b*, 3*b*), which contain few or no noradrenergic afferents from the LC.

The β_2 -binding sites are less widely distributed and account for a smaller percentage of the total ^{125}I -PIN binding sites among most of the subnuclei of the IPN. Rostrally, the β_2 -binding sites are concentrated as a narrow band along the ventral border of the IPN (Fig. 2*c*). At this level, these binding sites amount to approximately one-third of the total ^{125}I -PIN binding within the rostral, central, intermediate, and dorsal subnuclei, but they account for more than half of the total labeling seen in the lateral subnuclei. This concentration is greater caudally where the β_2 -binding sites are densely distributed in the ventral half of these subnuclei (Fig. 3*c*). Optical density measurements indicate that β_2 -binding sites account for more than 70% of total ^{125}I -PIN binding sites through this ventral half of the lateral subnuclei [total ^{125}I -PIN binding, 73.2 ± 3.3 (SE); β_2 -binding, 53.3 ± 3.0 (SE) fmol/mg protein]. Finally, β_2 -binding sites are not obviously associated with the series of blood vessels that run through the intermediate subnuclei of the IPN.

The FRs also contain ^{125}I -PIN binding sites and these are primarily β_1 -binding sites. The majority of β_2 -binding sites near the FRs are largely restricted to labeling along the glia limitans (Fig. 4).

In summary, labeling is found among subnuclei that receive little or no noradrenergic innervation, as well as those that do receive noradrenergic projections. The densest concentration of ^{125}I -PIN binding sites occurs at the ventral border of the IPN, within the ventral half of the lateral subnuclei and represents primarily β_2 -binding sites. β_1 -Binding sites are found in all of the subnuclei of the IPN but are densest in the subnuclei that are the targets of LC afferents.

Effects of lesions

Area measurements

Measurements of the areas of each subnucleus from the IPN of control and lesioned animals show no significant difference among the groups at the levels from which the optical density measurements were made and at the time course examined (Table 2). These data indicate that the shrinkage resulting from

Figure 4. Radioautogram ^{125}I -PIN binding sites shows labeling of FR. *a*, Distribution of total ^{125}I -PIN binding sites. *b*, β_1 -Binding sites account for the majority of ^{125}I -PIN binding sites seen in the FR. *c*, Very little labeling of β_2 -binding sites is seen in the FR. IF, Interfasiculus nucleus.

Table 2. Area measurements of subnuclei of the interpeduncular nucleus ($\times 10^4 \mu\text{m}^2 \pm \text{SE}$)

Subnuclei	Control	BLC (acute)	BLC (chronic)	BFR (acute)	BFR (chronic)
Laterals	15.5 $\pm$ 1.2	13.5 $\pm$ 1.5	16.3 $\pm$ 1.3	13.5 $\pm$ 1.2	12.7 $\pm$ 1.8
Intermediates	08.5 $\pm$ 0.5	07.8 $\pm$ 0.7	08.8 $\pm$ 0.6	09.2 $\pm$ 0.8	11.1 $\pm$ 0.8
Central	30.3 $\pm$ 2.2	32.2 $\pm$ 2.8	31.2 $\pm$ 2.4	30.8 $\pm$ 2.2	20.4 $\pm$ 3.54

Unpaired *t* tests performed on measurements from the left and right sides of the lateral and intermediate subnuclei showed that there is no significant difference in area between sides for either control or any of the lesion groups. Therefore, the reported values are the averages of the 2 sides for these 2 subnuclei. Newman/Keuls multiple-range test revealed no significant difference for the areas of the subnuclei measured at the levels from where the optical density measurements were recorded. BLC, bilateral locus coeruleus lesion; BFR, bilateral fasciculus retroflexus lesion ($n = 3-8$).

denervation cannot account for the differences we see in ^{125}I -PIN binding density following partial deafferentation.

Changes in density of binding sites

Changes in the density of ^{125}I -PIN binding sites after partial deafferentation of the IPN were examined 1 week (acute) or 6 and 8 weeks (chronic) postoperatively. There was no difference in binding density between the 6 and 8 week experimental groups for either of the lesion paradigms (Student's unpaired *t* test), so these 6 and 8 week survivors were pooled as the chronic lesion group.

LC lesions. LC lesions remove the noradrenergic afferents to the central, intermediate, and rostral subnuclei of the IPN and result in an increase in the density of ^{125}I -PIN binding sites, primarily as a result of increases in the density of β_1 -binding sites (Fig. 3, *d-f*). This increase occurs throughout the rostral, central, intermediate, and lateral subnuclei and is statistically significant at the chronic lesion period (Fig. 5*a*). There is no change in density of β_1 -binding sites in the dorsal subnucleus (Figs. 3*e*, 5*a*).

An increased density of β_2 -binding sites occurs in the central, intermediate, and lateral subnuclei, and this increase is largely restricted to the ventral portions of these subnuclei (Figs. 3*f*, 5*b*).

FR lesions. FR lesions remove non-noradrenergic afferents, elicit an increased density of noradrenergic projections in the rostral, central, and intermediate subnuclei (Battisti et al., 1987), and result in changes in the density of ^{125}I -PIN binding sites in the IPN. The density of β_1 -binding sites decreases significantly in all of the subnuclei, except the dorsal subnucleus, by 1 week postoperatively. In the chronic lesion group, a further decrease in the density of β_1 -binding sites occurs in the central subnucleus and intermediate subnuclei that are the primary targets of the increased noradrenergic projection (Battisti et al., 1987) (Figs. 3, *g-i*; 6*a*).

The density of β_2 -binding sites is also changed after FR lesions. An increased density of β_2 -binding sites occurs in the rostral, central, intermediate, and lateral subnuclei (Fig. 6*b*). This reflects primarily a marked increase in the density of these binding sites at the ventral border of the IPN. The greatest increase in density of β_2 -binding sites, however, occurs in the ventral half of the lateral subnuclei, at the caudal level of the IPN, where the greatest concentration of β_2 -binding sites is normally present (control, 53.3 ± 2.9 ; acute, 56.5 ± 2.3 ; chronic, 79.1 ± 1.3 ; $p < 0.05$, Newman/Keuls multiple range test; Figs. 3*i*, 6*b*).

Summary of lesion-induced changes. Both the β_1 - and the β_2 -binding sites exhibit changes in density, but no changes in the pattern of distribution, in response to deafferentation of the IPN. Following LC lesions, the β_1 -binding sites increase in density in

most of the subnuclei in which they are normally found. The density of β_2 -binding sites also increases in response to LC lesions.

FR lesions also cause changes in the density of β_1 - and β_2 -binding sites. The density of β_1 -binding sites decreases in response to these lesions by 1 week postoperatively (acute group). A further significant decrease in density of β_1 -binding sites occurs in the chronic lesion group in those subnuclei that are also targets of LC afferents. In contrast, the density of β_2 -binding sites increases in response to FR lesions, and with a slower time course than for the changes in β_1 -binding sites. The increase is generally confined to a narrow ventral border of the IPN.

Discussion

The IPN contains a large number of ^{125}I -PIN binding sites; the 2 subtypes, however, have quite different distributions. The β_1 -binding sites account for the majority of total ^{125}I -PIN binding sites in the rostral, central, and intermediate subnuclei of the IPN. The distribution of β_1 -binding sites is similar to the distribution of noradrenergic afferents from the LC to the IPN. β_1 -Binding sites are also found in the lateral subnuclei, which do not receive demonstrable noradrenergic innervation from the LC. β_2 -Binding sites account for most of the ^{125}I -PIN binding sites within the ventral portion of the lateral subnuclei and are densely distributed along the base of the IPN. Additionally, the FRs, the fiber bundles comprising major non-noradrenergic afferents to the IPN, contain β_1 -binding sites but few β_2 -binding sites. Our results also demonstrate plasticity of both the β_1 - and β_2 -binding sites in response to lesions, and this plasticity appears to be regulated differently for the 2 subtypes.

β_1 -Binding sites in the IPN increase in density following noradrenergic denervation. This increase in density of β_1 -binding sites in the normal target subnuclei of the noradrenergic LC, the rostral, intermediate, and central subnuclei is consistent with the classical idea of receptor "up-regulation"; i.e., the changes are related to denervation of noradrenergic input to the receptor.

Conversely, lesions of the FR increase the density of noradrenergic innervation to the IPN (Battisti et al., 1987) and β_1 -binding sites decrease in density in the rostral, central, and intermediate subnuclei. Since some of these β_1 -binding sites appear to be on FR axons, some of the decrease in β_1 -binding sites can be accounted for by degeneration of the lesioned FR axons. Decreases in the density of these binding sites continue to occur, however, in the central and intermediate subnuclei, beyond the time at which degenerating FR axons are seen (Murray et al., 1979). It is therefore likely that the continued decrease in density of β_1 -binding sites is related, at least in part, to the increased availability of NE to these subnuclei and the decreased β_1 -binding site density is consistent with "down-regulation."

A. β_1 binding sites B. β_2 binding sites

Figure 5. Changes in β_1 - and β_2 -binding site density in IPN subnuclei following LC lesions. Data were pooled for those subnuclei that are seen at both the rostral and caudal levels and in which no significant difference in binding density occurred between the 2 levels (unpaired Student's *t* test). No significant difference in β_1 -binding site density was found between the 2 levels for any of the subnuclei. β_2 -Binding site density was significantly different for the 2 levels of the lateral subnuclei ($p \leq 0.05$). These values, therefore, are reported separately as lateral subnuclei at the caudal level (Lat C) and lateral subnuclei at the rostral level (Lat R). *Acute*, 1 week postoperative; *Chronic*, 6 or 8 weeks postoperative. Binding density equals femtomoles bound/mg protein. Error bars indicate SE. *Significant difference compared with control values, $p \leq 0.05$ Newman/Keuls multiple-range test. **Significant difference between control, acute, and chronic time period, $p \leq 0.05$ Newman/Keuls multiple-range test. *A*, β_1 -Binding sites increase in density following LC lesions in most of the IPN subnuclei. There is no significant difference in density in the dorsal subnucleus. *B*, β_2 -Binding sites increase in density following LC lesions, except in the rostral and dorsal subnuclei.

Most β_1 -binding sites in the IPN appear, therefore, to be regulated homotypically by noradrenergic innervation. β_1 -Adrenergic receptors are defined, in part, by their affinity for NE (Lands et al., 1967) and the reciprocal changes in the density of the β_1 -binding sites that occur in LC target subnuclei can be correlated with the increased or decreased density of the noradrenergic projection.

The β_1 -binding sites in the lateral subnuclei also appear to be

A. β_1 binding sites B. β_2 binding sites

Figure 6. Changes in β_1 - and β_2 -binding site density in IPN subnuclei following FR lesions. Data were pooled for those subnuclei that are seen at both the rostral and caudal levels and in which no significant difference in binding density occurred between the 2 levels (unpaired Student's *t* test). No significant difference in β_1 -binding site density was found between the 2 levels for any of the subnuclei. β_2 -Binding site density was significantly different for the 2 levels of the lateral subnuclei ($p \leq 0.05$). These values, therefore, are reported separately as lateral subnuclei at the caudal level (Lat C) and lateral subnuclei at the rostral level (Lat R). *Acute*, 1 week postoperative; *Chronic*, 6 or 8 weeks postoperative. Binding density equals femtomoles bound/mg protein. Error bars indicate SE. *Significant difference compared with control values, $p \leq 0.05$ Newman/Keuls multiple-range test. **Significant difference between control, acute, and chronic time period, $p \leq 0.05$ Newman/Keuls multiple-range test. *A*, β_1 -Binding sites decrease in density following FR lesions in most of the subnuclei of the IPN. β_1 -Binding site density is not significantly different in the dorsal subnucleus. *B*, β_2 -Binding sites increase in density following FR lesions in most of the IPN subnuclei. There is no significant difference in density of sites in the dorsal subnucleus.

reciprocally regulated by changes in noradrenergic innervation, although little or no noradrenergic innervation has been demonstrated in these subnuclei (Hemmendinger and Moore, 1984; Battisti et al., 1987). In the periphery, β_1 -receptors have an equal affinity for NE and epinephrine (Lands et al., 1967), but there is also no evidence that epinephrine is present in the IPN (Hökfelt et al., 1974; Van der Gugten et al., 1976).

This response of the β_1 -binding sites in the lateral subnucleus to LC lesion, in view of the absence of direct noradrenergic or adrenergic innervation, is more difficult to interpret. The FRs contain β_1 -binding sites, suggesting that β_1 -binding sites are synthesized by medial habenular neurons and transported down

the FR axons to the lateral subnuclei of the IPN. Since lesions of the LC partially denervate the medial habenulae (Kobayashi et al., 1975), it is conceivable that a transynaptic effect could alter the synthesis of β_1 -binding sites. It has also been suggested that neurotransmitters can act at a distance (Chesselet, 1984; Schmitt, 1984; Herkenham, 1987), which could explain the frequent occurrence of apparent mismatches between binding sites and afferent distribution in the CNS. Changes in the density of β_1 -binding sites in the lateral subnuclei could therefore indicate a humoral regulation by NE diffusing to this area. Thus, the β_1 -binding sites could be regulated by noradrenergic input in an unconventional manner. Whatever the mechanism, our results are consistent with pharmacological studies that have shown β_1 -receptors responsive to alterations in noradrenergic availability in other areas of rat CNS (Minnemann et al., 1979a, 1982; U'Prichard et al., 1979; Levin, 1982; Dooley et al., 1983) and support the suggestion that the β_1 -receptors receive and are regulated by a noradrenergic input.

Previous studies were unable to detect alterations in the characteristics of β_2 -receptors following manipulations that altered the β_1 -receptors (Minnemann et al., 1979a, 1982; Nahorski et al., 1979; Levin, 1982; Dooley and Bittiger, 1987). This led to the suggestion that the β_2 -receptors are restricted to non-neuronal structures such as cerebral blood vessels and/or glia and are not regulated by noradrenergic neurons (Minnemann et al., 1979a, 1981). β_2 -Binding sites are associated with the ventral border of the IPN, and some of these binding sites are probably associated with the glia limitans and pia arachnoid. β_2 -Binding sites, however, do not appear to be associated with the 2 prominent rows of blood vessels that pierce the intermediate subnuclei of the IPN. We found no anatomical evidence, therefore, that the β_2 -receptors within the IPN are associated in any substantial numbers with blood vessels.

Our results show that β_2 -binding sites in the IPN increase in response to both FR and LC lesions. Glial cells would be expected to proliferate in the IPN in response to deafferentation and could account for the increase in β_2 -binding sites if these sites are present on reactive glial cells. It is possible that β_2 -binding sites within the lateral subnuclei are associated with neurons as well as glial cells. The distribution of β_2 -binding sites in the IPN correlates well with the distribution of neurons that are immunoreactive for several peptides. Galanin-immunoreactive terminals appear along the ventral border of the IPN, and the galanin-immunoreactive cell bodies are concentrated in the ventral half of the lateral subnuclei at the caudal level (Hamill et al., 1986b). In addition, serotonin-immunoreactive cell bodies, as well as substance P and substance K immunoreactivity, are similarly found in these caudal ventrolateral aspects of the IPN (Hamill et al., 1984; Hemmendinger and Moore, 1984; Artymyshyn and Murray, 1985; Murray et al., 1988). We cannot, therefore, rule out the possibility that the β_2 -binding sites are regulated by non-noradrenergic neurochemicals, such as peptides. Examples of this kind of heterotypic coregulation exist for several other systems (Tassin et al., 1982, 1987; Fuxe et al., 1983; Herve et al., 1986; Scott and Crews, 1986).

Receptors, in the mammalian brain, do not always match the distribution of their afferents (reviewed by Herkenham, 1987). The IPN contains areas where the β -adrenergic receptors match the distribution of their afferents and other areas where they do not. The distribution of β_1 -binding sites correlates best with the distribution of noradrenergic afferents, and our results support the suggestion that β_1 -binding sites are regulated by the avail-

ability of NE. β_2 -Binding sites do not correspond with the distribution of noradrenergic afferents in the IPN. We have shown, however, that β_2 -binding sites also respond to changes in innervation within the IPN, although they appear to be regulated by different mechanisms than the β_1 -binding sites.

Binding sites for several other receptor systems are densely distributed in the IPN (opiates: Artymyshyn et al., 1987; nicotinic and α -bungarotoxin: Hamill et al., 1986a; tachykinins: Murray et al., 1988; muscarinic and α -bungarotoxin: Rotter and Jacobowitz, 1984). Studies of the effect of lesions on these binding sites, however, showed no detectable change in the density of these binding sites in some of the IPN subnuclei (Clarke et al., 1986; Artymyshyn et al., 1987; Murray et al., 1988) and a decrease in the density of these sites in other IPN subnuclei, which probably indicates a presynaptic location on the lesioned axons (Price et al., 1984; Clarke et al., 1986; Artymyshyn et al., 1987). There is, therefore, no convincing evidence of either up-regulation or down-regulation for opiate, cholinergic, or tachykinin binding sites in the IPN in response to lesion. Our studies demonstrate both up-regulation and down-regulation of the β -adrenergic binding sites in response to lesions. This may imply a greater plasticity of the β -adrenergic binding sites within the CNS.

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