A series of electron microscopic immunocytochemical studies was performed to analyze subcellular sites for noradrenergic modulation in monkey prefrontal cortex. One out of 12 noradrenergic varicosities, identified by dopamine β-hydroxylase immunocytochemistry within single ultrathin sections, forms morphologically identifiable junctions with small dendrites and spines. Accordingly, α2-adrenergic receptors, almost all of which are of the A-subtype, that occur in spines are localized discretely over postsynaptic membranes. α2-Adrenergic receptors are also found at sites along axons, dendritic shafts and astrocytic processes lacking morphologically identifiable synaptic junctions, suggesting that these receptors are activated by volume transmission. In particular, axonal α2-adrenergic receptors occur mostly at pre-terminal regions, suggesting that axo-axonic interactions may mediate reduction of neurotransmitter release at sites other than axo-spinous junctions by closing voltage-dependent calcium channels. These results indicate that noradrenergic modulation of prefrontal cortex involves synaptic interactions at spines of pyramidal neurons and non-synaptic volume transmission to glia, dendritic shafts and axons.

Introduction

Area 46 of the prefrontal cortex integrates sensory, mnemonic and motor components of behavior that are guided by memory of visual-spatial stimuli. Abnormality in the prefrontal cortex and areas connected to it can lead to many mental disorders, including attention deficit hyperactivity disorder, Korsakoff’s amnesia and schizophrenia (reviewed by Goldman-Rakic, 1987). Noradrenergic innervation and the consequent activation of α2-adrenergic receptors (AR) in the prefrontal cortex is an important component of prefrontal cortical function. In non-human primates, decrease of noradrenergic innervation to the prefrontal cortex, either by ageing or chemical lesion, causes deficits in visuospatial memory of delayed response task, a prefrontal cortical function (Arnsten and Goldman-Rakic, 1985) and systemic infusion of α2-AR agonists ameliorates this deficit (Arnsten and Goldman-Rakic, 1985; Arnsten et al., 1988). Furthermore, the prefrontal cortex is the sole cortical region with a reciprocating projection to the locus coeruleus-noradrenergic system. Thus, generation of maintained attention, or vigilance, by the locus coeruleus-noradrenergic system (reviewed by Aston-Jones, 1985) may be regulated potently by polysensory memory which, in turn, is derived from prefrontal cortical input to the locus coeruleus.

Classic works examining noradrenaline release in the periphery and brain have shown that α2-AR serve in the negative feedback at noradrenergic synapses by promoting closure of voltage-dependent calcium channels located on noradrenergic axons, thereby reducing noradrenaline release (reviewed by Chesselet, 1984). On the other hand, improvement brought about by exogenous noradrenaline or α2-AR agonists can persist even after significant loss of noradrenergic fibers. This is an indication that noradrenergic receptors may reside on other non-noradrenergic axons that interact with noradrenergic axons. Yet another possibility is that α2-AR reside on dendrites, somata or astrocytes.

In this study, we sought to determine the precise site of noradrenergic modulation by the electron microscopic localization of noradrenergic axons. Noradrenergic axons were identified using an antisera directed against the noradrenaline-synthesizing enzyme, dopamine β-hydroxylase (DBH), as done previously for light microscopy (Lewis and Morrison, 1989). In addition, since our preliminary electron microscopic results indicated that synaptic partners of noradrenergic axons in the prefrontal cortex are not always obvious, sites of noradrenergic action were determined also by electron microscopic immunocytochemical identification of the α2A-, α2B-, and α2C-AR.

Materials and Methods

Source of Antisera and Description of their Specificity

Rabbit anti-DBH was purchased from Eugene Tech. Biotinylated goat anti-rabbit IgG was purchased from Vector and gold-labeled goat anti-mouse IgG was purchased from Amersham. Rabbit antisera directed specifically against the A, B and C subtypes of α2-AR were produced and characterized previously (Kurose et al., 1993). These receptors were produced by inoculating rabbits with glutathione-S-transferase (GST)-fusion proteins corresponding to the third intracellular loop portion of the human receptor molecules. All antisera were affinity-purified to eliminate GST immunoreactivity. The antisera were shown to recognize a single band on Western blots of COS cell membranes expressing the respective subtype of α2-AR and to immunoprecipitate only the appropriate subtype expressed in COS cells. Preadsorption of the antisera with the synthetic peptides used for inoculation eliminates the immunoreactivity within aldehyde-fixed brain tissue. Further details of their specificity are described elsewhere (Kurose et al., 1993; Aoki et al., 1994).

Preparation of Tissue

Brains of three Macaca fascicularis were generously provided to us by Dr Keith P. Purpura of Cornell University Medical College, where animals are housed according to Animal Welfare Assurance No. A3290–01. In addition, the prefrontal cortical area of one Macaca mulatta was generously provided to us by Drs Alev Erisir and Murray Sherman of SUNY Stony Brook. The brains of M. fascicularis were fixed with aldehydes by transcardial perfusion with the following solutions: (i) 500 ml of heparinized saline, delivered at a rate of 70–90 ml/min; (ii) a mixture of 5% acrolein and 4% paraformaldehyde buffered with 0.1 M phosphate buffer (PB) (pH 7.4) and delivered at a rate of 70–90 ml/min; (iii) 4% paraformaldehyde buffered with 0.1 M PB (pH 7.4), delivered at a rate of 70–90 ml/min for 6 min, then at a rate of 10 ml/min over a 50 min period. The brain of M. mulatta was fixed by transcardial perfusion with 3 l of heparinized saline, followed by 4 l of a mixture of 4% paraformaldehyde and 0.1% glutaraldehyde.

All brain tissue was postfixed in 4% paraformaldehyde solution for at least 1 day. Dorsolateral prefrontal cortical blocks centering around Walker’s area 46, i.e. dorsal and ventral to the principal sulcus and
anterior to the arcuate sulcus, were sectioned at a plane perpendicular to the principal sulcus using a vibratome set at a thickness of 40 µm. Some of the fibers appear to be of thicker caliber (arrowheads). Small single arrows point to short segments of varicose fibers that appear within the plane of section. Large open arrows point to pial surface. (B) α2A-AR immunoreactivity occurs as puncta over perikarya (arrowheads) as well as in fine processes that appear astrocytic (small arrows). (C) β-AR immunoreactivity, shown at a higher magnification, occurs as large (arrowhead) and finer (small arrows) punctate labeling associated with perikarya. (D) β-AR immunoreactivity is homogeneously distributed throughout the neuropil. In addition, immunoreactive puncta (small arrows) occur aggregated over perikarya, some of which are pointed to with curved arrows. Asterisks point to nucleoplasm that are relatively unlabeled. Bar = 50 µm in A and B, 25 µm in C and D.

Figure 1. Light microscopic localization immunoreactivity for dopamine β-hydroxylase, α2A-AR and β-AR. (A) DBH-immunoreactivity is localized to varicose fibers that course tangentially (small double arrows) and radially (small triple arrows). Some of the fibers appear to be of thicker caliber (arrowheads). Small single arrows point to short segments of varicose fibers that appear within the plane of section. Large open arrows point to pial surface. (B) α2A-AR immunoreactivity occurs as puncta over perikarya (arrowheads) as well as in fine processes that appear astrocytic (small arrows). (C) α2A-AR immunoreactivity, shown at a higher magnification, occurs as large (arrowhead) and finer (small arrows) punctate labeling associated with perikarya. (D) β-AR immunoreactivity is homogeneously distributed throughout the neuropil. In addition, immunoreactive puncta (small arrows) occur aggregated over perikarya, some of which are pointed to with curved arrows. Asterisks point to nucleoplasm that are relatively unlabeled. Bar = 50 µm in A and B, 25 µm in C and D.

Light Microscopic Immunocytochemistry
Free-floating sections were processed for immunocytochemistry by the avidin–biotin–peroxidase complex procedure (ABC) as described in detail previously (Aoki et al., 1993). The concentration of antisera were the following: 1:1000 with the addition of 0.05% Triton X-100 for the anti-DBH antiseraum; 1:100–1:200 for the A, B and C subtypes of α2A-AR. Sections were incubated with one of these antisera under constant agitation for 1 day at room temperature or 3 days at 6°C. The sections were stored until use at 6°C in 0.01 M PB containing 0.9% sodium chloride and 0.05% sodium azide.

Electron Microscopy
Upon confirmation by light microscopy that sections were optimally immunolabeled, those sections to be analyzed for electron microscopy were processed further using conventional methods. In brief, they were postfixied with osmium tetroxide, then with uranyl acetate dissolved in 70% alcohol, then flat-embedded by sandwiching between two sheets of Aclar plastic using EMBED812 (Electron Microscopic Sciences) as resin. The flat-embedded sections were re-examined under the light microscope to choose regions for ultrastructural analysis. Chosen areas spanned the entire thickness of cerebral cortex and showed no traces of red blood cells in blood vessels. These were re-embedded in Beem capsules. Sections were ultrathin sectioned at planes tangential to the surface of vibratome sections at a thickness of 70–90 nm and examined with and without lead citrate counterstain using a JEOL 1200XL electron microscope.

Semi-quantitative Electron Microscopic Analysis
Care was taken to analyze portions of the ultrathin sections that were immediately at the interface of tissue and the embedding matrix, where penetration by immunoreagents would be maximal. The immunolabeled profiles were identified as one of the following: axon, dendritic shaft, dendritic spine, astrocytic process or unidentifiable. Axons were identified by the presence of vesicles and/or neurofilaments. Axons which exhibited five or more vesicles and local swelling were considered varicosities. Dendritic shafts and spines were identified by the presence of postsynaptic densities and the absence of vesicles but, instead, smooth endoplasmic reticulum of irregular sizes and shapes. Dendritic shafts contained numerous microtubules and mitochondria and were >500 nm
in diameter. Dendrites with diameter ≤ 500 nm in diameter and lacking mitochondria or microtubules were considered spines.

The immunolabeled neuronal profiles were evaluated further for being synaptic or not by determining whether peroxidase reaction product accumulated along the presynaptic plasma membrane or the vesicles of the axon terminals forming morphologically identifiable synapses or along the postsynaptic membrane of dendrites. These profiles were tagged as 'synaptic and immunolabeled'. The frequency of encounter with each of these profiles was normalized to the total number of immunoreactive profiles encountered for each layer of each specimen of monkey prefrontal cortex. In addition, the frequency of encounter with immunolabeled synapse (at pre- or postsynaptic side) was determined by computing the ratio of labeled synapses to total number of synapse encountered (labeled + unlabeled) and of the labeled synapses to the area (in µm²) surveyed.

Results

Light and Electron Microscopic Characteristics of Noradrenergic Axons
Axons were identified as noradrenergic by immunocytochemical visualization of the noradrenaline-synthesizing enzyme, DβH. Light microscopy showed that morphological features of DβH-immunoreactive fibers in the specimen were indistinguishable from those described earlier (Lewis and Morrison, 1989). Specifically, the DβH-immunoreactive fibers were varicose, coursed tangentially in layer 1 and more radially in layers 2–6 (Fig. 1A). These fibers were densest in layer 5.

Electron microscopic examination of the DβH-immunoreactive fibers revealed that these contain numerous small clear vesicles within enlarged portions that are ~500 nm in diameter. These were considered varicosities. Immunoreactivity was associated with vesicular membranes clustered in varicose portions of axons more than in narrower, presumably intervvaricose, segments containing fewer vesicles. DβH-immunoreactive varicosities formed asymmetric synaptic junctions, where the postsynaptic membranes were associated with identifiable postsynaptic densities (Fig. 2A). The formation of symmetric synaptic junctions was also evident by the parallel alignment of the plasma membranes belonging to the terminal and to the dendrite adjacent to it and by the clustering of vesicles in the terminal towards the adjacent dendrite (Fig. 2B). Typically, the synaptic junctions were not encapsulated by astrocytic processes.

Semi-quantitative analysis was performed upon prefrontal cortical tissue obtained from two monkeys: 374 DβH-immunoreactive profiles were encountered from all six layers of one tissue and 190 from the other. For both specimens, portions forming morphologically identifiable synapses averaged ~9%, or ~1 out of every 12 DβH-immunoreactive axonal profiles encountered within single ultrathin sections of 70 nm thickness. One notable exception to this pattern occurred in layer 6, where none of the 79 immunoreactive axonal profiles formed morphologically identifiable synaptic junctions.

α2-AR Localization
Immunocytochemical labeling of the A-, B- and C-subtypes of α2-AR in the prefrontal cortex indicated that the A-subtype is
overwhelmingly predominant. By light microscopy, \( \alpha_{2A} \)-AR-immunoreactivity was present in all layers, including layer 1. Immunoreactivity was concentrated in neuronal perikarya where they occurred as fine puncta (Fig. 1B,C). Both larger, presumably pyramidal, and smaller, presumably GABAergic interneuronal, perikarya were immunoreactive. Fine grains of immunoreactivity also occurred in the neuropil. Fine wispy processes, suggestive of astrocytic processes, also were immunoreactive. In contrast, the immunoreactivities of the B- and C-subtypes were subthreshold for detection by light microscopy (not shown), while \( \beta \)-AR immunoreactivity was more prevalent over somata and neuropil (Fig. 1D). By electron microscopy, the B- and C-subtypes were detectable but very sparsely and in such low amounts within each immunoreactive profile that differentiation from background was difficult (Fig. 3). Where detectable, immunoreactivity for the B- and C-subtypes occurred over postsynaptic densities and along extrajunctional membrane of dendrites and axons. Due to their sparsity, the immunoreactivity for the B- and C-subtypes were not subjected to further ultrastructural analysis.

Electron microscopy showed that perikarya labeling for \( \alpha_{2A} \)-AR, seen by light microscopy, reflects association of these receptors with the Golgi apparatus, rough endoplasmic reticulum and nonsynaptic portions of the plasma membrane (not shown). Almost all of the axosomatic synapses were devoid of immunoreactivity for \( \alpha_{2A} \)-AR, even for cases where immunoreactivity was evident elsewhere within perikarya.

Immunoreactivity occurred directly over postsynaptic membranes of dendritic spines forming asymmetric synaptic junctions (Figs 4A,B and 5C,E) or of dendritic shafts forming symmetric synaptic junctions (Fig. 4C). In some cases, portions of the plasma membrane beyond the synaptic cleft (Figs 4A and 5E) were also immunoreactive. Labeled portions of spines lacking morphologically identifiable synaptic junctions were invariably near terminals.

Axons were immunoreactive for \( \alpha_{2A} \)-AR at portions exhibiting and lacking morphologically identifiable synaptic junctions. Within portions of axons with morphologically identifiable synapses, immunoreactivity was clearly associated with presynaptic plasma membranes (Fig. 5A,C) as well as the membranes of small clear (Fig. 5A,C) and dense-cored (Fig. 5F) vesicles. Axon terminals directly juxtaposed to other terminals (Fig. 5B,F) and axons (Fig. 5D) were also immunoreactive. Within labeled preterminal portions, immunoreactivity was again associated with both vesicular and plasma membranes. Some fortuitous planes of section allowed verification that immunoreactivity could occur in preterminal portions even when immunoreactivity was not apparent (Fig. 5B,D) or was barely at threshold for detectability (Fig. 5E) in terminal portions containing larger clusters of vesicles. Most of the junctions formed by the \( \alpha_{2A} \)-AR-immunoreactive terminals were asymmetric. Occasionally, astrocytic processes coursing in the vicinity of immunolabeled axons and dendrites were immunoreactive (Fig. 5E).

For semi-quantitative analysis, 7985 \( \alpha_{2A} \)-AR-immunoreactive profiles were encountered across the six cortical layers from two monkey prefrontal cortices. The analysis showed that, in every layer, \( \alpha_{2A} \)-AR-immunoreactivity was more prevalent in axons than in dendrites. Specifically, 37% of all immunoreactive profiles encountered were axonal, while 23% were immunoreactive at dendrites. On the other hand, while the portion of immunoreactive axons exhibiting immunoreactivity at morphologically identifiable synapses was low (14% of 2928 immunoreactive axonal profiles encountered), the corresponding value for dendrites was high (54% of 1852 immunoreactive dendrites encountered), yielding a 3-fold greater frequency of \( \alpha_{2A} \)-AR-immunoreactivity at postsynaptic sides of morphologically identifiable synapses. A substantial portion of \( \alpha_{2A} \)-AR-immunoreactivity occurred in astrocytic profiles (26%).

Discussion

General Methodological Issues

This study was embarked on to determine the cellular and subcellular sites of noradrenergic action within intact prefrontal cortex of monkeys. To this end, noradrenergic axons and \( \alpha_{2} \)-subtypes of noradrenergic receptors were visualized immunocytochemically so as to be able to determine their subcellular localization. Antisera used in this study were extensively characterized for their specificity in previous studies by Western blots and immunoprecipitation of biologically active

Figure 3. Ultrastructural localization of \( \alpha_{2B} \)-AR and \( \alpha_{2C} \)-AR immunoreactivity. (A) \( \alpha_{2B} \)-AR immunoreactivity is barely detectable along the postsynaptic membrane (arrowhead) and extrajunctional portions (small arrow) of a spine that is postsynaptic to a terminal (T). An unidentifiable small profile in its vicinity (asterisk) is also immunoreactive. (B) \( \alpha_{2C} \)-AR immunoreactivity is barely detectable along a nonjunctional portion of a terminal (small arrow within T). Arrowhead points to an unlabeled postsynaptic density associated with the labeled terminal. Bar = 500 nm for both panels.
whole molecules (Kurose et al., 1993; Aoki et al., 1994). Preadsorption controls using aldehyde-fixed brain sections were also performed for the receptor antibodies (Aoki et al., 1994; Venkatesan et al., 1996). Nevertheless, quantitative analysis performed for the present study indicated that the areal density of immunoreactive profiles is highly variable across blocks from different monkeys. This may reflect the fact that immunodetection depends heavily on the efficiency of tissue fixation, duration of primary antibody incubation and/or on the titer of the primary antibody. For this reason, we encountered difficulty finding a consistent laminar distribution pattern of the profiles immunoreactive for noradrenergic receptors and axons. On the other hand, the relative frequency of immunoreactive profiles (i.e. axonal vs. dendritic vs. astrocytic) was more constant across blocks from different monkeys. This is most likely because these measures are independent of the efficiency of immunolabeling, so long as the sampling is done close to the surface of tissue where penetration by immunoreagents would be optimal. Thus, measurements of the relative frequency of immunolabeled profiles were deemed more reliable.

The present results complement earlier studies using in situ hybridization to determine population of neurons expressing noradrenergic receptor mRNAs. Comparisons of our data with earlier data describing $\alpha_{2A}$-AR mRNA expression in rat cerebral cortex indicate similarities and some differences. Specifically, our immunocytochemical results indicated immunoreactivity within cell bodies in all layers of the cerebral cortex, while results from in situ hybridization indicated that $\alpha_{2A}$-mRNA is expressed prominently in layer 6, to a lesser degree in layers 2–4, and little if at all in layer 5. It is possible that layer 5 neurons express very low levels of $\alpha_{2A}$-mRNA and these levels would become detectable by lengthening the period for autoradiographic exposure. Alternatively, there may be species differences in the probe or in cortical organization leading to differences in the results obtained by the two methods. Indeed, results obtained from yet another method, i.e. receptor autoradiography, has shown $\alpha_2$-AR binding sites in all layers of the monkey cerebral cortex, just as is seen with our immunocytochemical detection of $\alpha_{2A}$-AR.

Discussed below are functional implications of the immunocytochemical findings.

**Subcellular Sites of Noradrenergic Action as Indicated by DH-immunoreactivity**

**Methodological Considerations**

Various anti-DH antisera have been used in the past for identifying noradrenergic axons in the cerebral cortex, including the prefrontal cortex of monkeys (Lewis and Morrison, 1989). Although this enzyme occurs in the synthetic pathway of adrenaline as well as noradrenaline, adrenergic fibers are not present in the cerebral cortex (Hökfelt et al., 1974). Thus, one could presume that DH-immunoreactive fibers are, indeed, noradrenergic. We observed that DH-immunoreactive fibers were highly varicose, coursed tangentially in layer 1 but were more varied in their orientation in the other layers. These features are in close agreement with earlier descriptions for noradrenergic fibers in the cerebral cortex of monkeys (Lewis and Morrison, 1989) and rats (reviewed by Fallon and Loughlin, 1987).

In order to preserve plasma membranes for ultrastructural analysis, we minimized the concentration of Triton X-100, a permeabilizing reagent most widely used for DH-immunocytochemistry. We noted that immunocytochemical reactivity was not detectable below a concentration of 0.05% (v/v). Fortunately, ultrastructural preservation with 0.05% Triton X-100 was adequate for identification of vesicles, asymmetric and symmetric synaptic junctions as well as dendritic, spinous, axonal and astrocytic profiles, as long as the tissue was well preserved by transcardial perfusion with aldehydes and post-fixation with osmium tetroxide and uranyl acetate.

![Figure 4. Postsynaptic localization of $\alpha_{2A}$-AR immunoreactivity. (A) $\alpha_{2A}$-AR immunolabeling is evident directly over the postsynaptic density of a dendritic spine (arrowhead) as well as the extrajunctional portion (small arrow) of the labeled spine (LS). The profile to its right, containing numerous vesicles, is the presynaptic axon terminal (T). Unlabeled spine (US), unlabeled postsynaptic density (open arrow points to its perforation) and unlabeled terminals (T) are shown for comparison with the labeled profile. (B) Within the spine shown here, immunoreactivity is heavily clustered over one portion of the postsynaptic density (arrowhead) and the nearby saccule (small arrow). Faint immunolabeling also is evident over the spine apparatus (sa). The presynaptic terminal contains numerous, uniformly sized, round vesicles and is devoid of immunolabeling. An unlabeled spine (US) is indicated for comparison. (C) $\alpha_{2A}$-AR immunoreactivity also is detectable in dendritic shafts, such as the one shown here (LD). In this example, immunoreactivity is discretely localized to the postsynaptic density (arrowhead). The terminal that is presynaptic to this labeled junction (T) contains pleomorphic vesicles, including one that is dense-cored (small arrow), and forms a junction with another dendrite exhibiting postsynaptic density of intermediate thickness but no immunolabeling (UD). Bar = 500 nm.
fibers within rat cortex, showing that ∼ is entirely consistent with earlier reports on the noradrenergic forms morphologically identifiable synaptic junctions. This value axons encountered within a single plane of ultrathin section analysis indicated that ∼ noradrenaline than of L-glutamate, thereby allowing for chemical astrocytic processes (Aoki and Kabak, 1992). The absence glutamatergic synapses, which are almost always surrounded by morphologically identifiable target. On the other hand, these found within single ultrathin sections form synaptic junctions. Our findings support this idea, since most of the noradrenergic receptors, particularly those along dendrites and axons, are also found at sites lacking morphologically identifiable synapses (see discussion below) but were invariably near a terminal.

Predominance of the A-subtype of α2-AR in the Prefrontal Cortex

While molecular biology has revealed the existence of three subtypes of α2-AR, the functional significance of this diversity has remained unclear, particularly since pharmacological tools are not yet available for selectively activating a single subtype.

What is so far most notable about the three subtypes is their differential distribution across tissue in the periphery (reviewed by Harrison et al., 1991; Bylund, 1992) and in brain (Nicholas et al., 1993; Scheinin et al., 1994). The present result expands this concept, showing that the monkey prefrontal cortex expresses the A-subtype almost exclusively. This contrasts with the Purkinje cells of the monkey cerebellum, where the B-subtype is expressed in high levels (Go et al., 1992). The prevalence of the A-subtype in the prefrontal cortex is in agreement with previous behavioral studies using performance in delayed response tasks to examine prefrontal cortical function (reviewed in Arnsten et al., 1996). These studies showed that guanfacine, which has relatively higher affinity for the A-subtype than for the B- and C-subtypes, also is more effective than the agonists preferring the B and C-subtypes in ameliorating prefrontal cortical deficits caused by noradrenaline depletion.

α2AR Is Most Prevalent in Axons

In the periphery and in the cerebral cortex of rats, α2-AR has been demonstrated to operate as an autoreceptor. Following release, noradrenaline binds to α2-AR located on axons. This leads to closure of voltage-dependent calcium channels, causing reduction in further release of noradrenaline (reviewed in Chesselet, 1984). Based on the prevalence of α2AR on axons, the present data indicate that α2-AR in the monkey prefrontal cortex could also be operating to reduce neurotransmitter release. The present study did not attempt to determine whether the transmitter content of α2AR-immunoreactive axons might be noradrenergic. Activation of α2-AR has been reported also to inhibit the release of serotonin as well as noradrenaline (Frankhuyzen and Mulder, 1980; Maura et al., 1982). These findings, together, suggest that axonal α2AR-immunoreactivity may reflect the presence of autoreceptors as well as heteroreceptors that regulate (probably inhibit) the release of noradrenaline and other neurotransmitters in the prefrontal cortex.

The great majority of immunoreactivity in axons was in preterminal portions. This preterminal staining was not likely to have resulted from diffusion of the peroxidase reaction product from terminal regions, since preterminal staining sometimes occurred without terminal staining. A likely function for such receptor localization is to prevent leakage of neurotransmitters at sites other than the most terminal portions.

Noradrenergic Receptors In Spines Are Highly Localized Over Postsynaptic Membranes

After axons, the most prevalent localization for α2AR was at dendritic shafts. However, synaptic localization was more prevalent within spines than in dendritic shafts or axons, because nearly all labeled spines exhibited discrete localization
of peroxidase reaction product over postsynaptic membranes. This predominantly spinous localization of synaptic labeling matches the synaptic innervation pattern of noradrenergic axons. Together, these results indicate that pyramidal neurons of the prefrontal cortex, which are spiny, receive noradrenergic neuromodulation via activation of α₂-AR at their spines.

Since noradrenergic axons were not identified in the same sections immunolabeled for the receptors, we cannot know whether immunoreactive synapses on spines are immediately postsynaptic to them. An alternative possibility is that the asymmetric synapses with immunoreactivity are glutamatergic (Aoki and Kabak, 1992), while the symmetric synapses are
Possible Role of Non-junctionally Localized Receptor
Immunoreactivity in Volume Transmission

Most α2-AR in proximal portions of neurons is at non-junctional sites and removed from the plasma membrane. Since these receptors cannot interact with extracellularly released noradrenaline, they probably reflect the receptor molecules undergoing turnover — i.e. de novo synthesis, desensitization and proteolysis. In support of this idea, immunoreactivity occurred adjacent to organelles involved in protein synthesis, such as the Golgi apparatus and endoplasmic reticulum. On the other hand, the immunoreactive patches localized along the plasma membrane but removed from synaptic junctions may reflect participation of the receptors in volume transmission. Thus, the extrasynaptic labeling for noradrenergic receptors may be an indication that synthetically released noradrenaline also diffuses slightly beyond synaptic clefts and activates extrajunctional noradrenergic receptors. This mode of neuromodulation may be more effective for regulating synaptic efficacy of a group of neurons during the delay period of delayed-response tasks challenging the prefrontal cortex.

Notes

We are grateful to Drs Keith P. Purpura, Alev Erisir and Murray Sherman for providing us with monkey prefrontal tissue and Mona Lubin, Alice Elste and Dr Alev Erisir for reading the manuscript. This work would not have been possible without the expert technical assistance from Alice Elste and X.-Z. Song. For the photographic reproduction, we thank Dr Mian Hou. This study was supported by grants from the Human Frontier Science Program (RG-16/93) and NIH-EY08055 to C.A. Part of this work was presented at the 1994 Annual Meeting of the Society for Neurosciences (Venkatesan et al., 1994) and the Eighteenth Catecholamine Symposium held in 1996 (Aoki et al., 1997). Address correspondence to Chiye Aoki, Center for Neural Science, New York University, 6 Washington Place, New York, NY 10003, USA. Email: chiye@cns.nyu.edu.

References


Little has been reported on the postsynaptic effect of α2-AR in the prefrontal cortex. In the locus coeruleus, α2-AR reduces firing of neurons by activation of K-channels (Williams et al., 1991). However, this phenomenon has not been observed for the prefrontal cortex or any other cortical region (McCormick et al., 1991). On the other hand, behavioral studies demonstrate that noradrenaline improves function of prefrontal cortices that have been denervated of noradrenergic fibers. This finding suggests that receptors are located on sites other than noradrenergic axons, which could include synapses on dendritic spines, dendritic shafts activated by volume transmission or non-noradrenergic axons receiving axo-axonic influences. Recently, Sawaguchi and Kikuchi (1997) have observed that delay-related firing of prefrontal cortical neurons is suppressed by iontophoresis of an α2-AR antagonist, yohimbine. This finding has been confirmed by Li and Mei (1995), who have also seen enhancement of delay-related firing by systemic clonidine, an α2-AR agonist. It is most likely that these electrophysiological effects reflect activation of α2-AR at axo-spinous junctions in the prefrontal cortex which, in turn, are mostly excitatory.
neuronal activity related to working memory in monkeys. Presented at the 3rd Congress of the Federal Asian and Oceanian Physiological Societies. Abstract S9–3(0).


During the hour-long presentation, the audience was engaged and interested in the research presented on neuronal activity related to working memory in monkeys. The discussion covered various aspects of the role of noradrenaline in the central nervous system and its implications for the function of the noradrenergic system.