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β_2-Adrenergic receptors are colocalized and coregulated with "whisker barrels" in rat somatosensory cortex

(^{125}I-labeled pindolol/2-deoxy-D-glucose)

PETER VOS*, DAVID KAUFMANN†, PETER J. HAND‡, AND BARRY B. WOLFE§¶

*School of Medicine, Department of Pharmacology, †Institute of Neuroscience, ‡School of Veterinary Medicine, Department of Animal Biology and School of Medicine, Department of Neurology, University of Pennsylvania, Philadelphia, PA 19104; and §School of Medicine, Department of Pharmacology, Georgetown University, 3900 Reservoir Road, NW, Washington, DC 20007

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ABSTRACT **Autoradiography has been used to visualize independently the subtypes of β-adrenergic receptors in rat somatosensory cortex. β_2-Adrenergic receptors, but not β_1-adrenergic receptors, colocalize with "whisker barrels" in this tissue. Thus, each whisker sends a specific multisynaptic pathway to the somatosensory cortex that can be histochemically visualized and only one subtype of β-adrenergic receptor is specifically associated with this cortical representation. Additionally, neonatal lesion of any or all of the whisker follicles results in loss of the corresponding barrel(s) as shown by histochemical markers. This loss is paralleled by a similar loss in the organization of β_2-adrenergic receptors in the somatosensory cortex. Other results indicate that these β_2-adrenergic receptors are not involved in moment-to-moment signal transmission in this pathway and, additionally, are not involved in a gross way in the development of whisker-barrel array.**

The large whiskers on the lateral aspect of the rat snout, called mystacial vibrissae, are organized in a species-specific pattern consisting of five rows; designated A to E (1–3). Each row contains four to eight whiskers. Rats, like humans, have a neuronal representation in the somatosensory cortex of most external sensory input. This somatosensory "map" is physically distorted, with the majority of the available space dedicated to the facial region (4). Histologic studies have demonstrated that there are columnar cortical representations of the mystacial vibrissae that span layer IV of the somatosensory cortex. Due to their appearance in histologically prepared coronal sections, these representations are known commonly as "whisker barrels." When viewed in tangential sections these whisker barrels are roughly circular in cross section.

Electrophysiological studies recording single-unit activity after stimulation of individual identified whiskers have demonstrated a largely one-to-one correspondence between these cortical whisker barrels and the mystacial vibrissae (4). Discrete loci in the primary somatosensory cortex have been identified where neuronal activity increased in response to stimulation of one and only one vibrissae. Furthermore, no overlap was observed between cortical receptive fields for the vibrissae. Similar results have been reported using radiolabeled 2-deoxyglucose to measure metabolic activity in whisker barrels. When individual identified whiskers were stroked, a significant increase in local glucose utilization was observed in the corresponding region of the cortical representation (5, 6).

In addition to such experiments that identify and visualize a specific whisker barrel, by staining tangential sections of the cortex for the activity of the mitochondrial enzyme succinate dehydrogenase, it is possible to visualize simultaneously the entire whisker-barrel field array (2). The cortical barrel field array displayed in the adult rodent has five rows (rows A–E) and can be predictably modified by whisker-follicle removal in the neonatal rat (2, 7, 8). When whiskers are removed from the contralateral buccal pad of neonatal rats, the central representation observed in the adult is deranged in an isomorphic manner. For example, when the whisker follicles in row C (the middle row) are removed, the corresponding histochemically visualized anatomical representation of row C in the cortex is greatly diminished, if not absent (2, 8, 9). The postnatal period during which the development of the central representation is sensitive to peripheral manipulation lasts a few days after birth (2, 7, 8).

Regularity of organization and ease of anatomical verification make this a useful system for studying the development and regulation of sensory pathways. The fidelity of the one-to-one correspondence resulting in the accurate central reproduction of peripheral patterns implies a high degree of structural organization between numerous interacting elements. The elements of immediate interest to us were the intrinsic cortical β-adrenergic receptors (βARs). Previous work from this laboratory, using autoradiographic techniques (10), indicated that the subtypes of βARs were heterogeneously distributed in rat cortex. In particular, it appeared that in coronal sections at the level of the somatosensory cortex, β_2-adrenergic receptors (β_2ARs) may be preferentially associated with the whisker-barrel array. The combination of autoradiographic and histologic techniques permitted us to test this suggestion directly. In this study we addressed two questions: (*i*) Are cortical β_2ARs colocalized with a histochemical marker (succinate dehydrogenase) for whisker barrels in the cortex? (*ii*) Are the derangements observed in the succinate dehydrogenase-labeled cortical whisker-barrel array after neonatal whisker-follicle removal echoed by changes in the patterns of cortical β_2ARs?

METHODS

Flat Mounts for Tangential Cortical Sections. Eight- to 10-week-old Sprague–Dawley rats (Charles River Breeding Laboratories) were killed by decapitation. The brains were removed and bisected at the midline. The subcortical regions were dissected bluntly from the cortex. The cortices were flattened between two glass microscope slides with a 2-mm spacer between slides and frozen on dry ice. Tissues were stored at −70°C until needed. Frozen tissue was mounted onto a cryostat chuck and, at −10°C, 32-μm-thick sections were cut parallel to the pial surface. These sections were thaw-mounted onto gel/alum-coated slides and stored at

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Abbreviations: βAR, β_1AR, or β_2AR, β-, β_1, or β_2-adrenergic receptor, respectively; ^{125}I-pindolol, ^{125}I-labeled pindolol.

¶To whom reprint requests should be addressed.

−70°C. For most experiments, adjacent sections were processed alternatively for autoradiographic analysis of βAR labeling or for histologic visualization of succinate dehydrogenase activity.

Autoradiography. Frozen sections were warmed to room temperature after processing. Dried slides were then incubated in one of four solutions depending on the receptor(s) to be visualized according to the method of Rainbow *et al.* (10).

Total βARs. Sections were incubated at 23°C in 20 mM Tris·HCl containing 0.9% NaCl (Tris-saline buffer) for 60 min in the presence of 250 pM ^{125}I-labeled pindolol (^{125}I-pindolol; Sandoz Pharmaceutical). Sections were then washed at 0–4°C for three 15-min periods in Tris-saline buffer. Washed sections were dipped once in distilled water at 4°C to remove buffer salts and then rapidly dried on a slide warmer at 56°C. Dried slides were then apposed to LKB Ultrofilm for 24 hr. The exposed film was developed and the resulting autoradiograms were mounted onto glass microscope slides for inspection.

βAR subtypes. To visualize binding of ^{125}I-pindolol to β_1-adrenergic receptors (β_1ARs), the sections were processed as outlined above in an incubation medium containing both 250 pM ^{125}I-pindolol and 50 nM ICI 118,551 (ICI), a selective β_2AR antagonist. To visualize binding of ^{125}I-pindolol to β_2ARs, a selective β_1AR antagonist, ICI 89,406 (70 nM; ICI), was substituted for ICI 118,551.

Nonspecific binding. Sections were incubated with 250 pM ^{125}I-pindolol and 100 μM (−)-isoproterenol, a nonselective agonist at βARs.

Succinate Dehydrogenase Activity. Thaw-mounted frozen sections were prepared for succinate dehydrogenase histochemistry by the method of Nachlas *et al.* (11) as modified by Durham and Woolsey (8) with one important difference. Air-dried sections were incubated at 37°C for 1 hr in a 50 mM sodium phosphate buffer (pH 7.2) containing 0.55 mM nitro blue tetrazolium and 50 mM sodium succinate. Stained sections were then incubated in 10% (vol/vol) formalin for 1 hr. Unlike the previous protocols, the slides were removed and rapidly dried on a hot plate set at 56°C before dehydrating through a series of graded alcohols (75%–100% ethanol), defatting in xylene, and covering with a coverslip. The drying between fixation and dehydration was done to prevent the subsequent discoloration of sections due to oxidation observed by Killacky and Belford (2). This procedure permits the long-term storage of stained sections with minimal loss of histological resolution or subsequent oxidation.

Peripheral Deafferentation. Neonatal rat pups were operated on between postnatal day 0 (day of birth) and postnatal day 2. Rat pups were anesthetized by hypothermic anesthesia. By using a dissecting microscope, scissors, and forceps, the mystacial vibrissae and the underlying follicles were removed unilaterally (5). This permitted each rat to serve as its own control. The wounds were then packed with sterile gel foam. After surgery, pups were resuscitated and returned to their mother. Rats were then allowed to mature for 8–10 weeks whereupon they were sacrificed and the brains were removed for analysis. Two types of deafferentation were performed. "Total" lesions entailed unilateral removal of all mystacial vibrissae along the lateral aspect of the snout. "Row C spared" lesions entailed the unilateral removal of all vibrissae from the whisker pad with the exception of those in row C.

2-Deoxyglucose Utilization. Central 2-deoxyglucose metabolism driven by peripheral stimulation was measured as described (5). Briefly, a femoral artery and vein were cannulated in anesthetized (halothane) adult male rats. The surgical wound was infiltrated with 0.25% Marcaine hydrochloride, a long-acting local anesthetic, and the incision was sutured. With the exception of the most centrally located whisker in row C (C3), all vibrissae were clipped close to the skin to prevent their accidental activation. Either saline or propranolol (10 mg/kg) was administered i.p. 20 min prior to the start of whisker stimulation. Stimulation consisted of rostro-caudal stroking of the remaining whisker at a frequency of 4–5 Hz with a mechanical stimulator. Stimulation was begun in conscious animals ≈2 min before the injection of 40 μCi of 2-deoxy-D-[1-^{14}C]glucose (New England Nuclear; specific activity of 56 mCi/mmol; 1 Ci = 37 GBq) in 0.5 ml of sterile saline. Stimulation was then continued for 45 min. Arterial blood samples were withdrawn at regular intervals and measured for 2-deoxy[^{14}C]glucose and glucose concentrations. After stimulation, animals were sacrificed, the brain was removed, and the cortex was flattened between two silicone slides separated by spacers. The tissue was frozen and cut in serial tangential sections on a cryostat at a thickness of 20 μm. Sections were thaw-mounted onto coverslips, dried on a slide warmer at 65°C, and apposed to Kodak SB-5 single-emulsion x-ray film. Films were exposed for 10 days. On the image generated by the cortex contralateral to the stroked whisker, a single dark spot corresponding to the C3 barrel metabolic representation overlying the C3 barrel was seen (e.g., see figure 6A of ref. 5) but on the other side of the barrel field exposure was homogeneous (data not shown). The dark spot (stroked) as well as the other (unstroked) side were analyzed using a computerized image processing system (DUMAS) as described by Feingold *et al.* (12). For each barrel field at least six sections were analyzed and the values were averaged to obtain estimates of glucose utilization. Three separate experiments were performed. In each experiment one control and one propranolol-treated rat were examined.

Drug Treatments. *Propranolol.* Fourteen rats from one litter were treated *in utero* by implantation of an osmotic minipump (Alzet) into the mother on embryonic day 17. The minipump was precalibrated to give an infusion of 10 mg·kg^{-1}·day^{-1} of propranolol, a nonselective βAR antagonist. This dose, given to adult animals, has been shown to increase the density of βARs in rat cerebral cortex (13). Whiskers were removed unilaterally from all pups at postnatal day 0 as described above, using the row C spared protocol. Seven of the pups from this litter were continued on propranolol at 10 mg/kg (three times a day as subcutaneous injections) for 8 days after birth.

Clenbuterol. Fifteen rat pups were treated from birth to postnatal day 8 with subcutaneous injections of the selective β_2AR agonist, clenbuterol (10 mg·kg^{-1}·day^{-1}). This drug has been shown to selectively down-regulate β_2ARs in the cortex without a significant decrease in the density of β_1ARs (14). Whiskers were removed from eight of the rats on the day of birth.

All rats, both lesioned and unlesioned, treated and untreated, were allowed to mature for 8–10 weeks whereupon their brains were removed for histochemical and autoradiographic analysis.

RESULTS

In initial experiments, adjacent tangential sections from control unlesioned adult rats were used to produce histologically stained sections visualizing succinate dehydrogenase activity (Fig. 1*A*). Given the fact that 32-μm-thick sections were cut, and our experience of obtaining a maximum of seven sections per animal with identifiable whisker barrels, we estimate that the stratum of cortex that we were studying was 200–250 μm thick. This corresponds to previous measurements. By knowing the approximate localization of the barrel-field array, autoradiograms visualizing total βARs, as well as β_1- and β_2AR subtypes and nonspecific binding, were produced as described (Fig. 1 *B–F*). The sections used for autoradiograms were bracketed by sections stained for suc-

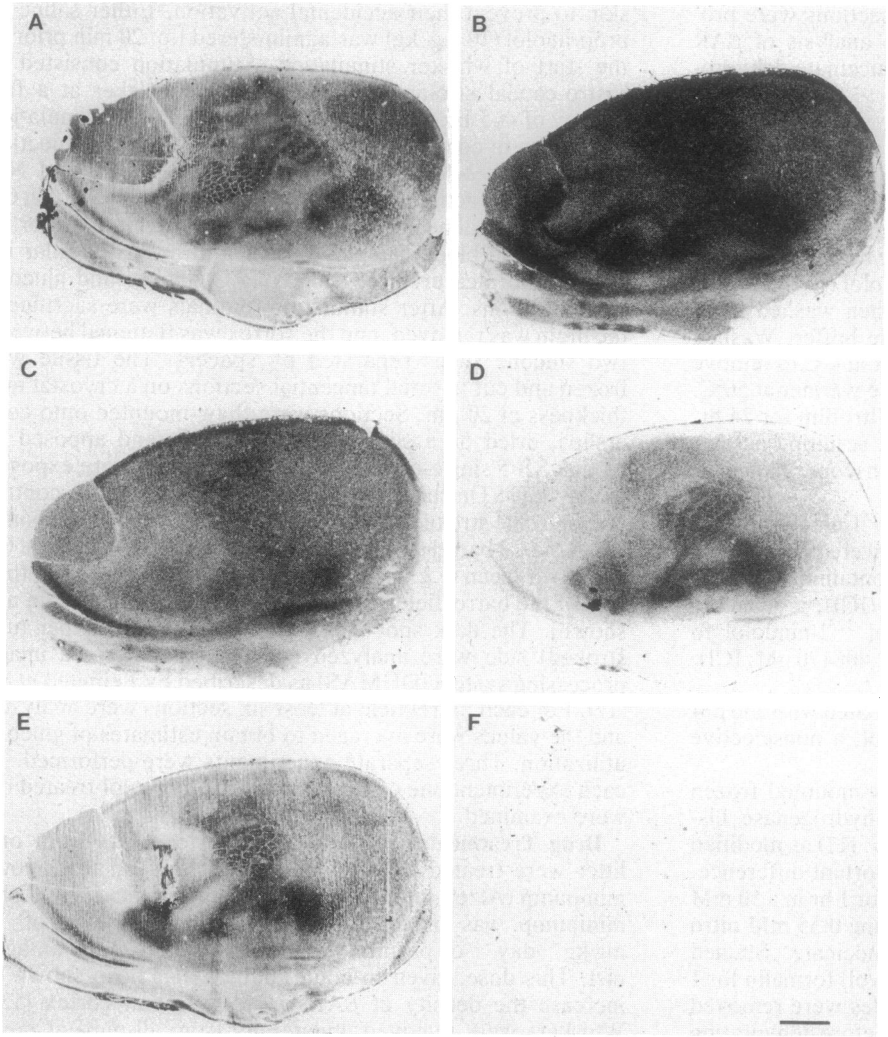

FIG. 1. Succinate dehydrogenase staining and βAR labeling patterns in control rat brain. Tangential sections from brain of control (8 week old) rat were prepared and labeled. (*A*) Succinate dehydrogenase staining. (*B*) Labeling of all βARs. (*C*) Labeling of β_1ARs. (*D*) Labeling of β_2ARs. (*E*) Succinate dehydrogenase staining. (*F*) Nonspecific binding. (Scale bar = 2.5 mm.)

cinate dehydrogenase activity. This ensured that the sections used for autoradiography were taken from layer IV of the cortex. The results from a representative experiment are shown in Fig. 1. The first section (Fig. 1*A*) was stained for succinate dehydrogenase activity and clearly visualizes the entire array of whisker barrels. The immediately adjacent section (Fig. 1*B*) was labeled with ^{125}I-pindolol in the absence of any other βAR ligands to visualize all of the βARs. This panel reveals an increased density of labeling in the general area of the whisker barrels but no clearly identifiable pattern. The next section (Fig. 1*C*), labeled with ^{125}I-pindolol in the presence of a β_2AR antagonist (ICI 118,551) to visualize the binding of radioligand to only β_2ARs, does not show a significant heterogeneity in binding throughout the section. The following section (Fig. 1*D*), which is labeled with ^{125}I-pindolol in the presence of an antagonist of β_1ARs (ICI 89,406) and visualizes binding to β_2ARs, clearly shows heterogeneity in the density of receptor labeling, as well as the distinctive pattern of the whisker-barrel array. The next sequential section (Fig. 1*E*) was stained for succinate dehydrogenase activity to verify that all sections were within the barrel field. The final section shown (Fig. 1*F*) demonstrates the low level of nonspecific binding. Similar results were obtained in >24 animals. Since the sections presented are sequential serial sections, the lack of a discernible pattern when labeling β_1ARs is not due to the section in question being outside of the barrel field. Thus, in the cortical barrel field, β_2ARs, but not β_1ARs, appear to be arranged in a pattern similar to that observed with staining for succinate dehydrogenase activity.

Peripheral deafferentation (i.e., removal of whisker follicles) during the "critical period" (from birth to a few days after birth) results in a derangement of the cortical representation that paralleled the removal of individual follicles (an isomorphic derangement), as visualized by histochemical means (2, 7–9). In other words, if the whisker follicles of row C (the middle row) on the whisker pad were removed during this time, the corresponding whisker barrels would be absent in the adult. To determine if changes in the barrel-field array caused by neonatal removal of follicles were associated with changes in the pattern of β_2ARs in this area, adjacent tangential sections from adult rats (8–10 weeks old) that had been unilaterally lesioned as neonates were labeled for either succinate dehydrogenase activity or β_2ARs. Frozen tangential sections were cut through the entire cortex. Every second section was stained for succinate dehydrogenase activity. The intervening unstained sections were prepared for autoradiographic visualization of β_2ARs.

Representative results are shown for three animals in Fig. 2. The sections were taken from levels with maximum pattern clarity. Fig. 2 *A* and *B* illustrates the patterns observed in the barrel field from a control unlesioned side. In these sections all five rows of mystacial vibrissae representations are visible and the correspondence of succinate dehydrogenase staining and β_2AR labeling patterns is clear. Fig. 2 *C* and *D* illustrates the patterns observed using the row C spared paradigm. The row C spared lesions were performed to test for the possibility of some nonspecific effect on the distribution of β_2ARs in the adult cortex after neonatal peripheral deafferentation. The observed succinate dehydrogenase staining (Fig. 2*C*) indicates

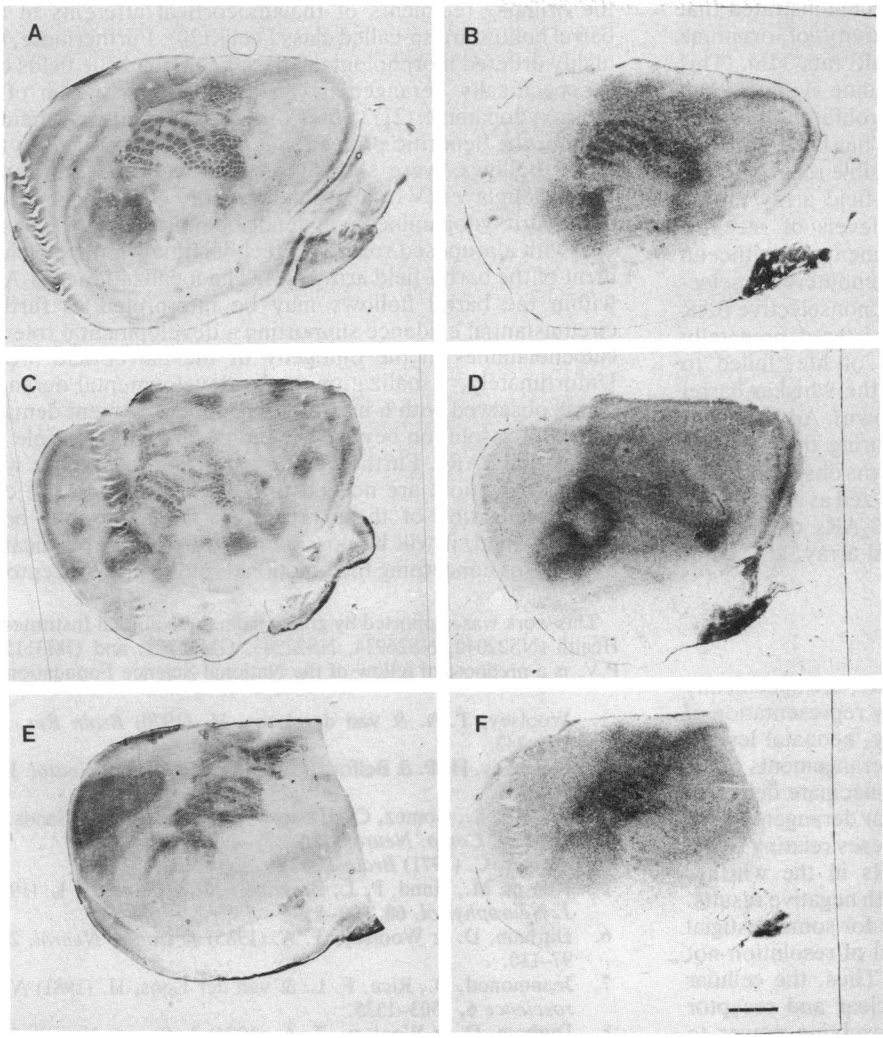

FIG. 2. Effects of lesions on succinate dehydrogenase staining pattern and β_2AR labeling pattern. Rats were lesioned during the first 2 days of life and allowed to reach maturity (8–10 weeks), and brains were removed, cut tangentially, and stained or labeled. (*A*, *C*, *E*) Succinate dehydrogenase staining. (*B*, *D*, *F*) β_2AR labeling. (*A* and *B*) Control unlesioned side. (*C* and *D*) Row C spared lesion. (*E* and *F*) Total lesion. (Scale bar = 2.5 mm.)

the single row of whiskers left intact are clearly represented in the cortex. Again, the pattern of β_2AR labeling is similar to the pattern of staining for succinate dehydrogenase. The adjacent rows that have been removed are visualized as fainter bands similar to those seen in the total-lesioned animals below. Fig. 2 *E* and *F* is representative of results obtained when all whiskers are removed from the contralateral whisker pad. The diffuse pattern of succinate dehydrogenase activity is paralleled by a diffuse pattern of β_2AR labeling. In these total-lesion animals, a consistent barrel-field array was never observed. A faint banding pattern similar to that described by Killacky and Belford (2) was the most that was observed. Comparison of either surgically lesioned cortex to the control cortex presented at top clearly shows that the intended lesion was successful in deranging the whisker-barrel array in the predicted manner. In sum, these results indicate that, using either succinate dehydrogenase staining or β_2AR labeling, central barrel field arrays are similarly deranged after peripheral deafferentation during the "critical period."

To learn something about the function of the β_2ARs in the whisker barrels, the ability of propranolol, a potent antagonist at βARs, to alter local glucose utilization stimulated by stroking the C3 whisker was examined. The question to be addressed was: Do β_2ARs mediate moment-to-moment signal transmission in cortical barrel fields? If the hypothesis was correct, it was expected that propranolol (10 mg/kg, i.p.) administered 20 min prior to administration of 2-deoxy-[^{14}C]glucose to a rat followed by continual stroking of the C3 whisker would alter the amount of 2-deoxy[^{14}C]glucose that is normally taken up in the corresponding whisker barrel. As can be seen in Table 1, stroking of the C3 whisker significantly stimulated uptake of 2-deoxy[^{14}C]glucose in both control and propranolol pretreated rats. There was, however, no significant difference on either the unstroked or the stroked side when control cortex was compared to propranolol-treated cortex. Thus, it appears that βARs are not critically involved in moment-to-moment signal transmission in the barrel-field array, at least as far as glucose metabolism is concerned.

Previous work (15) in the cat visual cortex has suggested that the plasticity of columns visualized with cytochrome oxidase staining is inhibited by prenatal or neonatal propran-

Table 1. Effect of propranolol on 2-deoxyglucose utilization in the C3 whisker barrel upon continual stimulation

	Glucose utilization, μmol per 100 g per min	
Treatment	Control	Propranolol
Unstroked	147 ± 16*	137 ± 5*†
Stroked	209 ± 19	187 ± 8†
Difference	62 ± 5	50 ± 13†

Data are mean ± SEM from three experiments. Values are for local cerebral glucose utilization. Stroked and unstroked values are statistically significant for both control and propranolol-treated tissue (*, $P < 0.05$ compared to stroked; paired *t* test). However, the differences between stroked and unstroked values were calculated for each animal and the means are not statistically significant when comparing control to propranolol-treated values (†, $P > 0.05$ compared to corresponding control; *t* test).

olol treatment. Additionally, it has been demonstrated that stimulation of β_2ARs increases the activity of ornithine decarboxylase in neonatal but not adult rats (16). This enzyme is the rate-limiting step in spermidine synthesis and has been implicated in rapid growth and proliferation of cells (17). Inhibition of ornithine decarboxylase has been shown to retard neural growth (18). To test the possible role of β_2ARs in the establishment of the adult barrel-field array during neonatal development, we altered the levels of receptor stimulation by prenatal and postnatal treatment with either an agonist or an antagonist at βARs. Both clenbuterol, a selective β_2AR agonist (14), and propranolol, a nonselective βAR antagonist (13), when chronically administered prenatally and postnatally during the first 8 days of life, failed to influence the pattern of development of the whisker-barrel field observed in the adult rat (data not shown). Additionally, lesioned rats treated with either drug during the pre- and postnatal periods displayed the same patterns observed in the lesioned, non-drug-treated rats when analyzed as adults (data not shown). Thus, it is not clear that β_2ARs control the general development of the whisker-barrel array, at least at a macroscopic level.

DISCUSSION

We have shown that β_2ARs, but not β_1ARs, are specifically associated with the central somatosensory representation of the rat's mystacial vibrissae. Additionally, neonatal lesions of the vibrissae that result in predictable derangements of the whisker-barrel pattern, as visualized by succinate dehydrogenase staining, are accompanied by similar derangements in the pattern of β_2AR labeling. Two hypotheses relating to the possible functional significance of β_2ARs in the whisker barrels have been developed and tested with negative results. This can be interpreted either as evidence for some vestigial role, a nonneural role, or a role at a level of resolution not detected with the techniques employed. Thus, the cellular localization of βARs in the brain is unclear and receptor autoradiography does not possess the resolving power to distinguish, for example, between one type of neuron and another or between neurons and glia.

The measurement of 2-deoxy[^{14}C]glucose utilization yields an indication of general metabolic activity within neurons. Since blockade of βARs by propranolol had no effect on stimulation of cerebral glucose utilization, it appears that the cortical β_2ARs are not involved in the central transmission of somatosensory information at least with respect to metabolic activity. Similarly, they appear not to be involved in a gross way in the development of the cortical barrel-field array since stimulation or blockade of the receptors during the "critical period" had no effect on the final pattern of barrels eventually seen in the adult. However, a more subtle developmental role for these receptors cannot be dismissed. As seen in Figs. 1 and 2, the receptors are clearly localized to the barrel hollows. The cytoarchitecture of the hollows and the effects of peripheral deafferentation upon their cellular components have been studied in detail. The hollows consist of thalamocortical afferents, stellate cell densities, as well as neuronal somas (2, 9, 19). Cytochrome oxidase labeling, another mitochondrial enzyme marker, has been shown to be localized to the barrel hollows and has been shown at the ultrastructural level to label primarily dendritic mitochondria (9). Given the similarity of succinate dehydrogenase staining and β_2AR labeling, it is possible that these receptors are located on the dendrites. In this context it is worth noting that the primary recipients of thalamocortical afferents in the barrel hollow are so-called class I cells (20). Furthermore, the highly ordered morphology of class I cell dendritic fields can be specifically deranged by neonatal administration of 6-hydroxydopamine (21). After 6-hydroxydopamine administration, the dendritic projections, normally restricted to the barrel hollows, were shown to be more diffusely arrayed throughout layer IV of the somatosensory cortex. This effect of 6-hydroxydopamine upon dendritic morphology is consistent with a proposed role for catecholamines in the development of the barrel-field array (22). The localization of β_2ARs within the barrel hollows may be interpreted as further circumstantial evidence supporting a developmental role for catecholamines in the ontogeny of the barrel-field array. Unfortunately, visualization of the developmental derangements observed with 6-hydroxydopamine treatment demand a level of resolution beyond the limits currently possible for visualizing βARs. Further studies, utilizing techniques with higher resolution, are needed to determine the precise cellular localization of these receptors. Once this has been accomplished, it will be possible to test more sophisticated hypotheses concerning the functional role of these receptors.

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