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9. In the cat the uncrossed ventral component of the corticospinal tract does not descend beyond thoracic levels. This partly explains the lack of retrograde transport through uncrossed pathways to the cortex contralateral to the lesion in group A cats. There is an uncrossed lateral component, however, and it is probably responsible for the occasional labeling of cells contralateral to the lesion in group A cats (< 40 cells per animal). The possibility exists, therefore, that the uncrossed corticospinal tract expands (or fails to retract) in response to neonatal hemisection. This possibility has not yet been explored. Preliminary degeneration studies demonstrate, however, that the crossed corticospinal tract grows around and descends caudal to the lesion site.
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Presynaptic Location and Axonal Transport of β_1-Adrenoreceptors in the Rat Brain

Abstract. *Interruption of the ascending noradrenergic neurons of the locus coeruleus in the rat forebrain with 6-hydroxydopamine produced a progressive accumulation, proximal to the lesion, of tritiated dihydroalprenolol binding activity over 2 days. This accumulation could be blocked by interrupting the neurons closer to their cell bodies. Competitive binding studies with the β_2 agonist Zinterol suggested that the accumulated β-receptors were primarily of the β_1 subtype. These results suggest that, in the rat brain, some β_1-adrenoreceptors are located in presynaptic, noradrenergic locus coeruleus neurons and are transported in their axons.*

The development of techniques for defining subtypes of various adrenoreceptors has led to a search for the cellular location and function of these receptors. For example, most studies point to a predominantly postsynaptic location of β_1-adrenoreceptors in the rat cerebral cortex, although it has been difficult to delineate the types of cells on which such receptors are located (*1*). Because cholinergic (muscarinic and nicotinic), cholecystokinin, and opioid receptors undergo axonal transport in presynaptic nerves (*2*), it seems that adrenoreceptors might also be transported in presynaptic noradrenergic neurons. Norepinephrine, its synthetic enzymes tyrosine hydroxylase and dopamine β-hydroxylase, and other proteins are transported in the ascending noradrenergic neurons of the locus coeruleus, which innervate the rat cerebral cortex (*3*). It is reported here that binding sites for the β-adrenoreceptor antagonist dihydroalprenolol (DHA) also move in these neurons, suggesting that β-adrenoreceptors are located in presynaptic neurons and undergo axonal transport in rat brain.

Adult male Sprague-Dawley rats (200 to 300 g) were injected stereotactically in the left forebrain (9.0 mm anterior to the intra-aural line, 2.0 mm left of the midline, and 8.0 mm below the skull's surface) (*4*) with 2 μl of 6-hydroxydopamine (6-OHDA) (4 μg/μl in distilled water containing 0.1 μg of ascorbic acid per microliter). Such injections produce a relatively selective interruption of the ascending noradrenergic neurons of the locus coeruleus and cause an accumulation of tyrosine hydroxylase and dopamine β-hydroxylase activities proximal to the lesion (*5*). The rats were decapitated at various times after the injections, and 2-mm-thick sections of anterior hypothalamus (AH) proximal to the lesion site (5.0 to 7.0 mm anterior to the intra-aural line) were removed and bisected into injected (left) and uninjected (right) halves (*5*).

In three preliminary experiments membrane fractions were prepared from AH sections pooled from uninjected rats and assayed for β-adrenoreceptor binding by the method of Torda *et al.* (*6*). The AH membranes (2 to 3 mg/ml, final concentration) were suspended in 75 m*M* tris-HCl (*p*H 7.7) containing 25 m*M* $MgCl_2$, and seven different concentrations (0.5 to 40 n*M*, final concentration) of [^{3}H]DHA (45.0 Ci/mmole, New England Nuclear) were added to a total volume of 150 μl. Incubation of triplicate samples was carried to equilibrium at 35°C for 15 minutes and terminated by vacuum filtration on Whatman GF/C filters with four washes of ice-cold 0.9 percent saline. The filters were dried and the radio activity was counted by liquid scintillation spectrometry with 33 percent efficiency. Specific binding, determined with 10 μ*M* (−)propanolol, ranged from 45 to 60 percent.

In these control rats (eight to ten per experiment), [^{3}H]DHA bound to AH membranes with an affinity (K_d) of 7.2 ± 1.5 n*M* and a maximal density (B_{max}) of 120 ± 10 fmole per milligram of protein, as determined by Scatchard analysis of the binding data (*7*). Two days after the 6-OHDA injections, maximal [^{3}H]DHA binding proximal to the left forebrain injection sites was 35 percent higher ($P < .05$) than in comparable, uninjected right AH (Fig. 1). This increase in binding was primarily attributable to an increase in the density of β-receptors, since there was a decrease in the apparent binding affinity on the lesioned side. Thus the increased receptor density proximal to the lesions appeared to be due to the accumulation of mobile β-adrenoreceptors, that is, to anterograde axonal transport. This increase in binding proximal to the lesion site was linear over a maximum of 2 days (the increase was 25 percent at 24 hours), after which the difference from the controls remained constant until 5 days.

The apparent accumulation of β-receptor binding activity could have been attributable to increased numbers of receptors formed in postsynaptic hypothalamic cells as a result of denervation caused by the lesions. To evaluate this possibility, ten rats were injected simultaneously with 6-OHDA in the left forebrain and the left median forebrain bundle in the posterior hypothalamus (4.0 mm anterior to the intra-aural line, 2.0 mm left of the midline, and 8.0 mm below the skull's surface). Two days later the rats were decapitated. Such injections interrupt ascending noradrenergic neurons from brainstem nuclei and block axonal transport of norepinephrine, its synthetic enzymes, and other proteins in ascending locus coeruleus neurons (*3, 5*). The injections prevented the accumulation of [^{3}H]DHA binding activity proximal to the more distal forebrain injection site (B_{max} for

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the left and right sides, 44.9 ± 7 and 46.0 ± 4.4 fmole of 30 n*M* [^{3}H]DHA per milligram of protein, respectively). These results suggest that axonal transport and not denervation hypersensitivity was responsible for the accumulation of β-receptors proximal to the left forebrain lesions, since the more proximal lesions appeared to block the axonal transport of receptors.

To define the subtype of β-adrenoreceptor undergoing axonal transport, AH membranes were pooled from groups of 8 to 12 rats that had received bilateral injection of 6-OHDA into the forebrain 2 days before and were assayed for [^{3}H]DHA binding in the presence of various concentrations of the β_2-adrenoreceptor agonist Zinterol HCl (Mead-Johnson) and 100 μ*M* guanosine 5′-triphosphate (*8*). The resulting curvilinear Hofstee plots (Fig. 2) were analyzed by an iterative, nonlinear computer program (*1*, *8*), which showed that AH membranes from control rats contained 56.8 ± 4.6 percent β_2-adrenoreceptors and 43.2 ± 2.5 percent β_1-adrenoreceptors. In the rats with forebrain lesions the high density of β-receptors proximal to the lesion appeared to be primarily attributable to an accumulation of β_1-receptors (B_{max} for rats with and without lesions, 105 ± 15 and 55 ± 8 fmole of [^{3}H]DHA per milligram of protein, respectively) ($P < .005$), since there was no significant difference in the density of β_2-receptors in the AH of these brains.

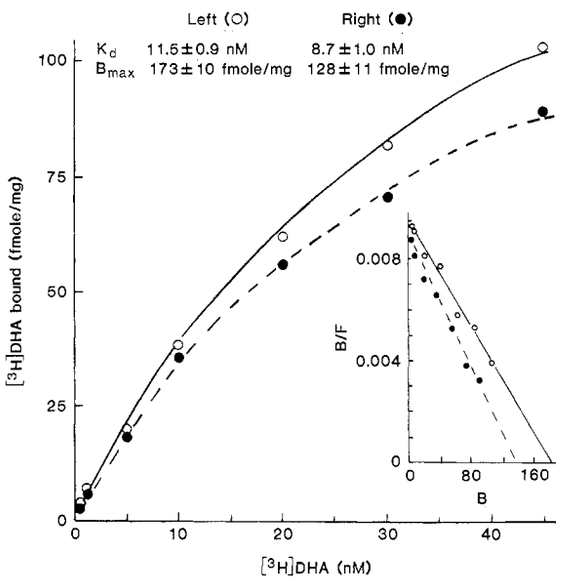

Fig. 1. Saturation isotherm showing the specific binding of [^{3}H]DHA to AH membranes from rats in which ascending noradrenergic neurons of the locus coeruleus were interrupted by 6-OHDA injections into the left forebrain 2 days before decapitation. Membranes from the injected (left) and uninjected (right) hypothalamic hemisections were incubated to equilibrium in the presence of seven different concentrations of [^{3}H]DHA. Values are means for three experiments carried out in triplicate. Specific binding was determined by using 10 μ*M* (−)propanolol to define nonspecific binding. Inset: Scatchard plot (*7*) of the data. *B* is [^{3}H]DHA bound (femtomoles per milligram of protein) and *F* is free concentration of the radioligand. The apparent K_d and B_{max} values derived from this analysis are given in the upper left-hand corner. The values for the left hemisections differ significantly from those for the right hemisections ($P < .05$, two-tailed paired Student's *t*-test).

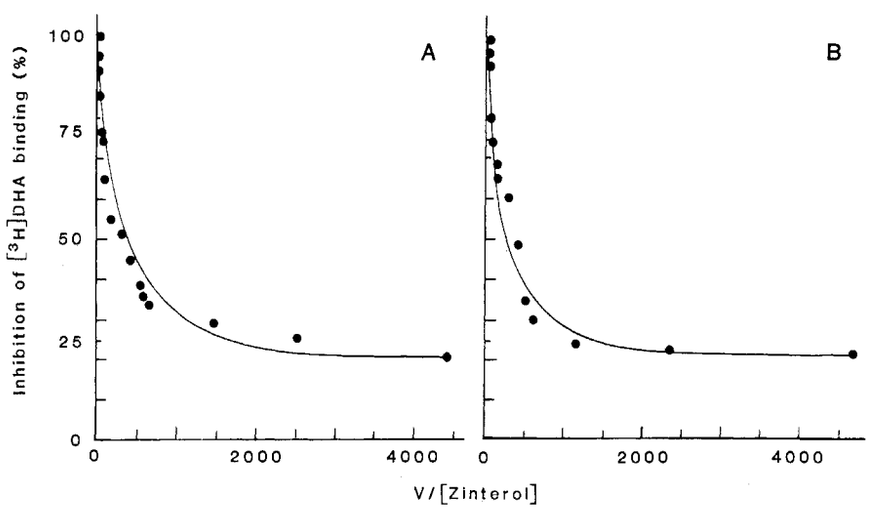

Fig. 2. Inhibition of [^{3}H]DHA binding to AH membranes from control rats (A) and rats injected in the forebrain (bilaterally) with 6-OHDA 2 days before decapitation (B). Membranes pooled from hypothalmic sections of 8 to 12 rats were incubated in the presence of 8 n*M* [^{3}H]DHA, 10^{-5} to 10^{-9} n*M* Zinterol, and 100 μ*M* guanosine 5′-triphosphate. The amount bound (expressed as the percentage of [^{3}H]DHA binding inhibited) is plotted on the ordinate, and this value, divided by the concentration of Zinterol, is plotted on the abscissa (*8*). Values are means for four to five experiments done in triplicate.

These results and those from previous studies (*3*, *5*) suggest that β_1-adrenoreceptors are transported in axons of noradrenergic neurons whose cell bodies lie in the locus coeruleus and whose terminals innervate the rat cerebral cortex. Because all β-receptors in the hypothalamus are not mobile and because there may be turnaround, subsequent retrograde transport, and loss of receptors through degradation at the lesion site, an accurate estimate of the transport rate could not be made. Nevertheless, the results do suggest that β_1-adrenoreceptors are mobile and that they reside in presynaptic noradrenergic neurons. Since destruction of cerebral cortical noradrenergic neurons with 6-OHDA leads to an increase rather than a decrease in the number of β_1-receptors, the evidence favors a postsynaptic location for at least some of these receptors (*1*). These data do not preclude the presence of presynaptic β_1-receptors, however, since a compensatory increase in the number of postsynaptic receptors could mask the concomitant loss of presynaptic receptors.

As in other studies in which various manipulations produced changes in β-adrenoreceptor densities (*1*), this study shows that β_1- but not β_2-receptors appear to be altered in the rat forebrain by such procedures and that β-receptors are found on a specific cell type. While the function of such presynaptic β_1-receptors remains to be elucidated, peripheral presynaptic β-receptors have been shown to facilitate stimulus-evoked norepinephrine release (*9*). These results should encourage similar studies in brain to define the functional role of presynaptic β_1-receptors in the control of noradrenergic neurons.

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10. I thank B. Van Ness for manuscript preparation, M. B. Finnegan for technical assistance, and R. O'Brien for computer analysis of the data. Supported by the Medical Research Service of the Veterans Administration and by a grant from Hoffmann–La Roche Inc.

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Adrenal Medullary Enkephalin-Like Peptides May Mediate Opioid Stress Analgesia

Abstract. *Different patterns of foot shock activate opioid and nonopioid mechanisms of stress analgesia in the rat. Opioid, but not nonopioid, stress analgesia is reduced by adrenal demedullation and denervation and is potentiated by reserpine, a drug known to increase concentrations of adrenal medullary enkephalin-like peptides. It is suggested that adrenal enkephalins mediate opioid stress analgesia.*

Enkephalins and larger enkephalin-like peptides are synthesized and stored, along with catecholamines, in chromaffin granules of the adrenal medulla (*1, 2*). Although the functional role of these opioids is not known, the fact that they are released by sympathetic activation or trauma (*2, 3*) suggests that they serve in the adaptive response to stress. The perception of pain is normally adaptive, impelling and guiding the organism into appropriate defensive behavior. However, pain suppression might prove more adaptive under conditions of emergency, when attention to noxious stimuli could disrupt effective coping (*4*). In fact, certain stressors are now known to cause potent analgesia in rodents (*5*). We previously found that varying only the temporal pattern of foot shock stress can determine whether an opioid or nonopioid form of analgesia occurs (*6*). We now provide evidence that adrenal medullary enkephalin-like peptides play a significant role in mediating opioid stress analgesia.

Seventy-two male Sprague-Dawley rats (350 to 400 g) were maintained on a 12-hour light cycle. The rats were anesthetized with sodium pentobarbital (50 mg/kg, intraperitoneally) and subjected to adrenalectomy (N = 16), adrenal demedullation (N = 24), denervation of the adrenal medulla by celiac ganglionectomy (N = 8), or sham surgery (N = 24). For the remainder of the experiment, the adrenalectomized rats were given 0.9 percent saline to drink. Two weeks after surgery, the rats were exposed to prolonged, intermittent or brief, continuous foot shock during the dark phase of the light cycle. These stress regimens cause opioid and nonopioid analgesia, respectively (*6*). Prolonged foot shock was 20 minutes of intermittently applied 60-Hz sine-wave pulses (one 2.5-mA, 1-second pulse every 5 seconds); brief foot shock was 3 minutes of continuously applied 60-Hz sine waves (2.5 mA). Pain responsiveness was measured by the tail-flick method (*7*), with exposure to the radiant heat limited to 7 seconds. Baseline latencies were determined immediately before prolonged foot shock and 17 minutes before brief foot shock in order to keep the interval between baseline testing and the termination of stress constant for all subjects. Immediately after foot shock stress, pain responsiveness testing was resumed. Tail-flick trials were conducted for 9 minutes at 1-minute intervals and then at 2-minute intervals for three more trials. Each rat ultimately received both stress procedures, administered in a counterbalanced fashion and separated by 1 week.

One week after the second exposure to stress some rats from each group were tested for analgesic responsiveness to morphine (2.5 mg/kg, subcutaneously). Pain responsiveness (calculated as the mean latency measured in three tail-flick trials separated by 1 minute) was determined once before morphine injection and again 30, 60, 120, and 180 minutes after drug administration. The limit of exposure to radiant heat was extended to 16 seconds in these trials. Finally, the effect of adrenal demedullation or denervation on adrenocortical function was assessed by measuring serum corticosterone. Additional groups of 18 rats received adrenal demedullation, adrenal denervation, or sham surgery. Two weeks later, a time corresponding to that at which the behaviorally tested rats received their first analgesia test, animals from each group were decapitated and trunk blood was collected from nonstressed rats (N = 6) or immediately after prolonged (N = 6) or brief (N = 6) foot shock. Serum samples were stored and corticosterone levels were determined later by radioimmunoassay (*8*). All data were compared by analysis of variance, and Newman-Keuls tests were used for specific comparisons between groups (*9*).

Both prolonged and brief stress elicited potent analgesia in sham-operated rats (Fig. 1). The groups differed significantly in their response to prolonged stress ($P < .01$) but not to brief stress. Specific comparisons indicate that adrenalectomized, adrenal-demedullated, and adrenal-denervated groups all showed less analgesia after prolonged stress than the control group ($P < .01$ in

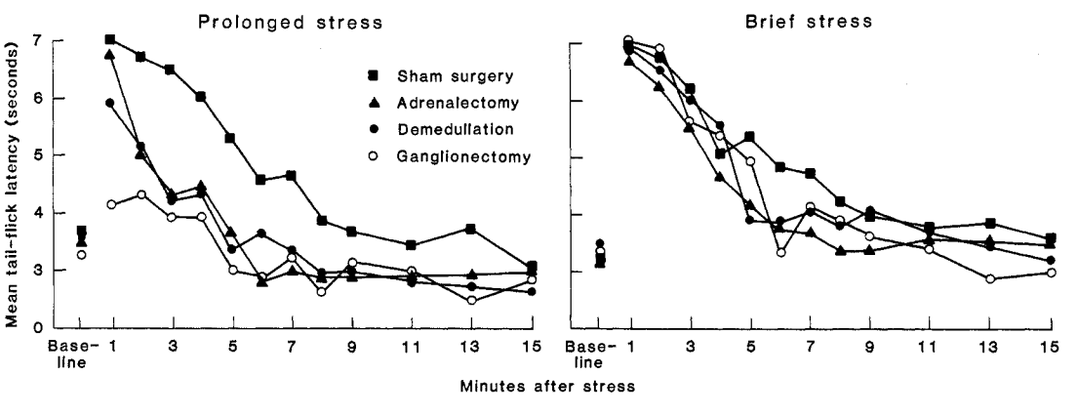

Fig. 1. Effects of adrenalectomy, adrenal demedullation, denervation of the adrenal medulla by celiac ganglionectomy, and sham surgery on the analgesic response to prolonged (opioid) and brief (nonopioid) foot shock. All three experimental procedures significantly attenuated opioid stress analgesia ($P < .01$) but not nonopioid stress analgesia.

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