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Axonal Transport of *Beta*-Adrenergic Receptors

Antero- and Retrogradely Transported Receptors Differ in Agonist Affinity and Nucleotide Sensitivity

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SUMMARY

Beta-receptors were measured in longitudinal sections of ligated rat sciatic nerve by autoradiographic localization of ^{125}I-labeled cyanopindolol binding sites. Receptors accumulated at the ligature, both proximally and distally, in a time-dependent fashion. Receptor transport also occurred in an isolated segment of nerve (i.e., a doubly ligated nerve), suggesting that the movement is by fast transport. Pharmacological analysis of the accumulating binding sites indicates that they are *beta*$_2$-adrenergic receptors. In competition studies, agonists were 10–30 times more potent on receptors accumulating proximal to the ligature than on distally accumulating receptors, whereas antagonists were equipotent on both. Guanyl-5′-yl-imidodiphosphate (GppNHp) decreased the potency of agonists at proximal receptors in a dose-dependent fashion. Distal receptors were much less sensitive to GppNHp. Other nucleotides displayed varying abilities to mimic the effect of GppNHp, suggesting the involvement of a guanine nucleotide-binding protein in regulating agonist affinity. Thus, presynaptic *beta*$_2$-adrenergic receptors were identified in rat sciatic nerve. A small fraction of them apparently moves by fast transport. The anterogradely transported receptors have binding properties which differ from the retrogradely moving receptor and appear to be in functional association with a nucleotide regulatory protein.

INTRODUCTION

Considerable physiological evidence (summarized in refs. 1–3) suggests that functional presynaptic *beta*-adrenergic receptors exist. At least in some systems, these *beta*-receptors appear to be involved in the potentiation of norepinephrine release. The *beta*$_1$ or *beta*$_2$ nature of the presynaptic receptors, as judged from physiological studies, may vary since in some systems it seems to be *beta*$_1$ (4) and in others *beta*$_2$ (5). In this communication, radioligand binding data are presented which suggest that presynaptic *beta*-adrenergic receptors exist in the rat sciatic nerve and that some of these receptors appear to be transported along the nerve. To identify the receptors, a high resolution, quantitative, *in vitro* autoradiographic technique (6) has been utilized.

Since the initial observation that opiate (7) and muscarinic cholinergic receptors (8) are present in axons and undergo axonal flow, the transport of other neurotransmitter receptors such as cholecystokinin (9), *alpha*$_2$-adrenergic,[3] H-1 histamine,[4] and insulin receptors has been detected. In all cases studied thus far, evidence suggests that the receptors move by fast transport, i.e., axonal transport. Also, we have recently reported (10, 11) that most of the anterogradely transported muscarinic receptors in the vagus nerve bind agonists with high affinity and are sensitive to modulation by GTP, whereas the retrogradely transported muscarinic receptors bind agonists with a much lower affinity and are less sensitive to modulation by GTP. Ions and nucleotides have been reported to regulate *beta*-receptors (12–14), and a model of the *beta*-receptor complex, composed of receptor, guanine nucleotide-binding protein, and adenylate cyclase, has been presented (15, 16). In this communication, the time course of *beta*-receptor accumulation at ligatures and the pharmacology and nucleotide sensitivity of the transported *beta*-adrenergic receptors are described.

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[3] J. R. Unnerstall, M. A. Zarbin, and M. J. Kuhar, in preparation.

[4] M. A. Zarbin, unpublished data.

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MATERIALS AND METHODS

Beta-adrenergic receptors were assayed by a highly reproducible and quantitative (6, 17, 18) *in vitro* autoradiographic technique which has been used in the assay of other transported receptors (7, 9–11). The details of the technique have been published elsewhere (6) and are outlined briefly below.

Tissue preparation. Male rats (150–250 g; Sprague-Dawley, Madison, Wisc.) were anesthetized with pentobarbital (65 mg/kg i.p.). The right thigh was then shaved and the underlying sciatic nerve was surgically exposed. The nerve trunk was dissected free of muscle and connective tissue and was ligated (4-0, silk nonabsorbable suture) at a point midway through the posterior compartment. When consecutive ligatures were placed on the nerve trunk, they were separated by 3–6 mm. During the dissection, care was taken to place as little tension as possible on the nerve trunk. The wound was closed with wound clips. After an appropriate ligation time, the nerve was surgically resected under anesthesia. Approximately 5 mm of nerve trunk were retained on either side of the ligature. A somewhat smaller length was retained distally so that the proximal nerve trunk could be identified histologically. The nerve was immediately mounted in brain paste (homogenized brain tissue) and frozen in liquid nitrogen on a brass microtome chuck. The mounted tissue was stored at −80° before sectioning. (Storage times as long as 5 days did not appear to affect receptor binding.) Frozen tissue sections (8 μm) of ligated sciatic nerve were cut on a Harris cryostat microtome (N. Billerica, Boston, Mass.) and were thaw-mounted onto subbed (chrome-alum) glass microscope slides. These tissue sections were stored at −20° and were used within 3 weeks of preparation.

Autoradiographic experiments. Beta-adrenergic receptors were labeled in slide-mounted tissue sections, and autoradiographs were generated by the apposition of emulsion-coated (Kodak, NTB3) cover slips against the slide-mounted tissue sections containing bound ^{125}I-CYP.[5] ^{125}I-CYP has been shown to be a ligand which binds to *beta*-adrenergic receptors with high affinity and high pharmacological specificity (19).

In preliminary experiments using slide-mounted tissue sections of rat brain and ligated rat sciatic nerve, it was found that, at a concentration of 50 pM, ^{125}I-CYP binding reached steady state after incubation for 120 min at 22°. Thus, incubations were routinely carried out for 150 min at 22°. The dissociation of specifically bound ^{125}I-CYP at 22° was very slow ($t_{1/2} > 1$ hr). Thus, wash times of 30 min at 22° were routinely employed to eliminate as much nonspecific binding as possible (no loss of specific ^{125}I-CYP binding was detectable at this time). The buffer used had the following composition: 50 mM Tris-HCl (pH 7.7), 0.01% ascorbate, and 5 mM $MgCl_2$. In other experiments, (±)-propranolol was found to have an IC_{50} of 10 μM in slide-mounted tissue sections of ligated rat sciatic nerve. Thus, binding in the presence of 10 μM (±)-propranolol was defined as nonspecific binding.

Quantitation of data. Data were quantified by counting autoradiographic grains in a Zeiss Universal microscope at ×100 magnification (oil immersion objective) using an eyepiece equipped with a superimposed grid. Enough grid squares were counted so that the average grain density per 95 μm^2 achieved a constant value (defined as a less than 10% change in the previous mean value). Because of variability in the tissue sections, it was necesssary to use consecutive tissue sections and to record grain densities in the same area of the nerve in all of the tissue sections derived from a given animal. This variability probably results from the fact that not all nerve fibers within ligated rat sciatic nerves exhibited ^{125}I-CYP binding sites. Previous studies (6, 17, 18) have shown that this autoradiographic technique is quantitative: grain density is proportional to exposure time and the tissue content of radioactivity. To ensure that the grain density was nearly linearly related to the concentration of radioactivity in the sample, exposure times yielding maximal grain densities less than 50 grains per 95 μm^2 were chosen. Thus, 4- to 6-day exposure times were usually employed. Nonspecific binding was usually 10–20% of total binding, depending on the age of the ligand. Specific binding is shown in all figures.

When analyzing the binding properties of transported *beta*-adrenergic receptors, only the area of the nerve lying 0.23 mm on either side of the ligature was assayed. It can be seen (Figs. 1 and 2) that this area contained a significant fraction of the receptor buildup, even after 27 hr of ligation. Accordingly, it was assumed that this area was enriched in transported binding sites.

Chemicals. ^{125}I-CYP (2000 Ci/mmole) was obtained from Amersham Corporation (Arlington Heights, Ill.). (±)-Cyanopindolol was the gift of Dr. Solomon Snyder. All other chemicals were obained from commercial suppliers.

RESULTS

Time course of receptor accumulation. In preliminary experiments, slide-mounted tissue sections of rat sciatic nerve were incubated with 50 pM ^{125}I-CYP to detect an accumulation of beta-adrenergic receptors at the ligature. The results of this experiment (Fig. 1) suggested that *beta*-receptors flowed along the nerve. To establish that the buildup of ^{125}I-CYP binding sites was due to a transport process, the time course of ^{125}I-CYP binding site accumulation was investigated. ^{125}I-CYP binding sites accumulated on both sides of the ligature in a time-dependent fashion (Fig. 2). During the first 13 hr of ligation, the grain density increased at a constant rate both proximal and distal to the ligature. At 27 hr of ligation, the rate of accumulation appeared to have decreased by approximately 40% both proximally and distally. By 96 hr, no further increase in grain density was detected proximal to the ligature, and a significant decrease in grain density was observed distally (compared with that at 27 hr).

In some experiments, we examined the saturation of ^{125}I-CYP to mounted tissue sections (13-hr ligation). The concentrations at which half-maximal saturation occurred were 100–150 pM, which is in reasonable agreement with biochemical studies where K_d values of 75–100 pM were measured under similar conditions.[4] Others (19) have found K_d values of 27–40 pM in various tissues. At near-saturating concentrations (700 pM), the relative accumulation between proximal and distal sites was the same as that shown in Fig. 2, indicating that the proximal-distal differences reflect differences in numbers of binding sites (data not shown).

Pharmacology of ^{125}I-CYP binding. To assess the pharmacological nature of transported ^{125}I-CYP binding sites, consecutive slide-mounted tissue sections of ligated rat sciatic nerve were incubated in solutions containing 50 pM ^{125}I-CYP or 50 pM ^{125}I-CYP plus various nonradioactive drugs. One micromolar practolol, *p*-aminoclonidine, and prazosin had no effect on ^{125}I-CYP binding wheras 1 μM (±)-propranolol was able to inhibit 80% of the ^{125}I-CYP binding proximally and distally (data not shown). These data are consistent with the notion that the ^{125}I-CYP binding sites are *beta*-adrenergic receptors (see also below).

Double-ligature experiments. Fast axonal movement, or axonal transport, can occur in an axon which is isolated from the cell body. To determine whether or not *beta*-receptor transport could occur in axons isolated from their cell bodies, ^{125}I-CYP binding was analyzed in nerve trunks around which two ligatures had been placed to create an isolated segment of nerve. An accumulation of *beta*-adrenergic receptors was detected proximal to both ligatures (Fig. 3), suggesting that isolated axons can

[5] The abbreviations used are: ^{125}I-CYP, ^{125}I-labeled cyanopindolol; GppNHp, guanyl-5′-yl-imidodiphosphate.

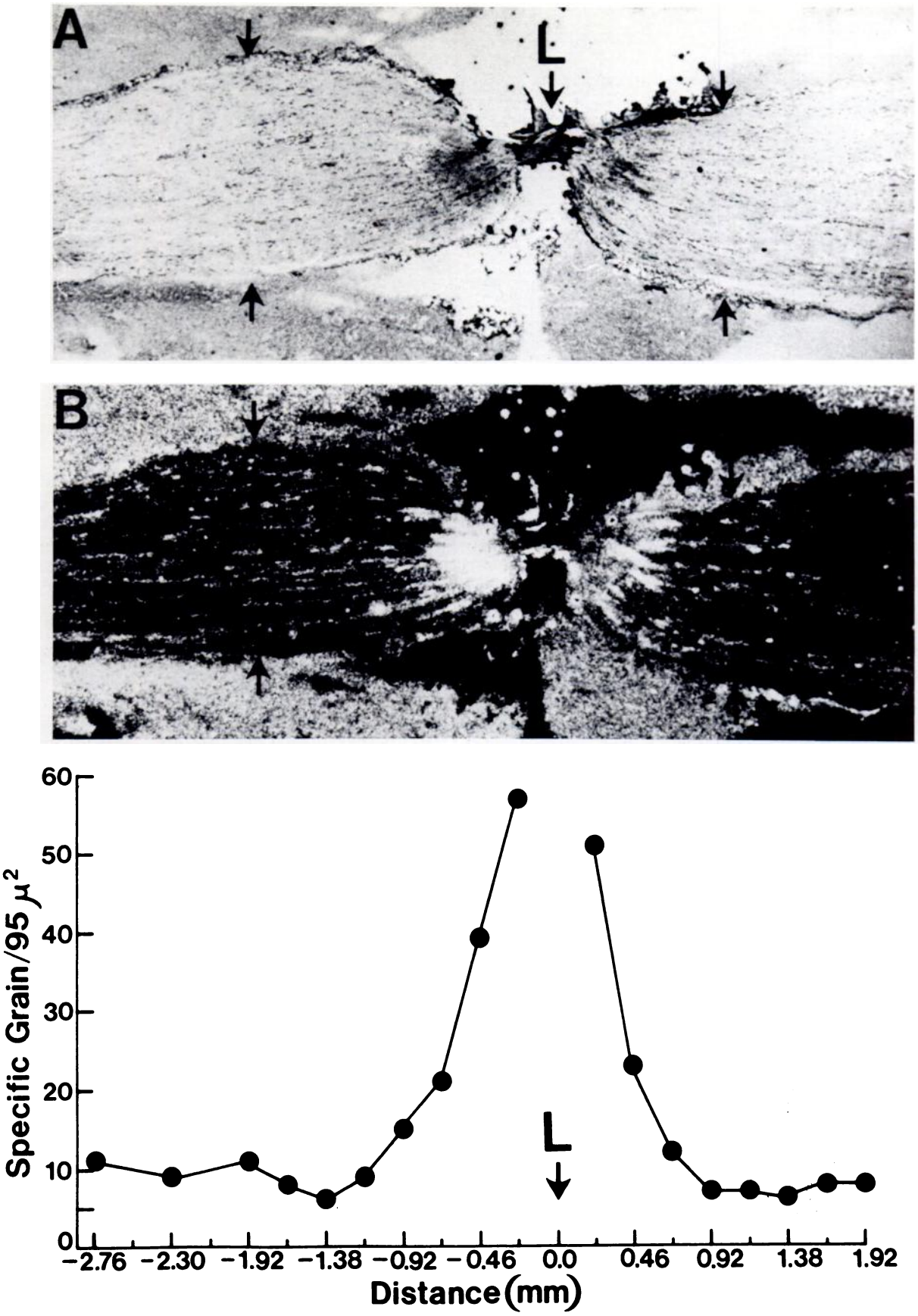

FIG. 1. *Autoradiographic localization of ^{125}I-CYP in a section of ligated sciatic nerve trunk*

A, A bright-field micrograph of the nerve trunk; B, a dark-field micrograph of the same slide. In B, the tissue is not visible, but the autoradiographic grain density is reflected by the relative brightness of the image; i.e., brighter regions have more receptors. The buildup of binding sites can be seen in B, both proximal and distal to the ligature, whose position is designated by *L* in A. The silk ligature itself fell off the slide during preparation. The pairs of *arrows* on either side of the ligature show the border of the nerve trunk. The tissue under the *arrows* and around the nerve is the brain paste in which the nerves were embedded.

Bottom, a plot of the grain density along the nerve. The buildup, both proximal negative numbers and distal to the ligature (*L*), is evident. The nerve was ligated for 18 hr. Exposure time of the autoradiogram was 6 days. See Materials and Methods and legend to Fig. 2 for additional details.

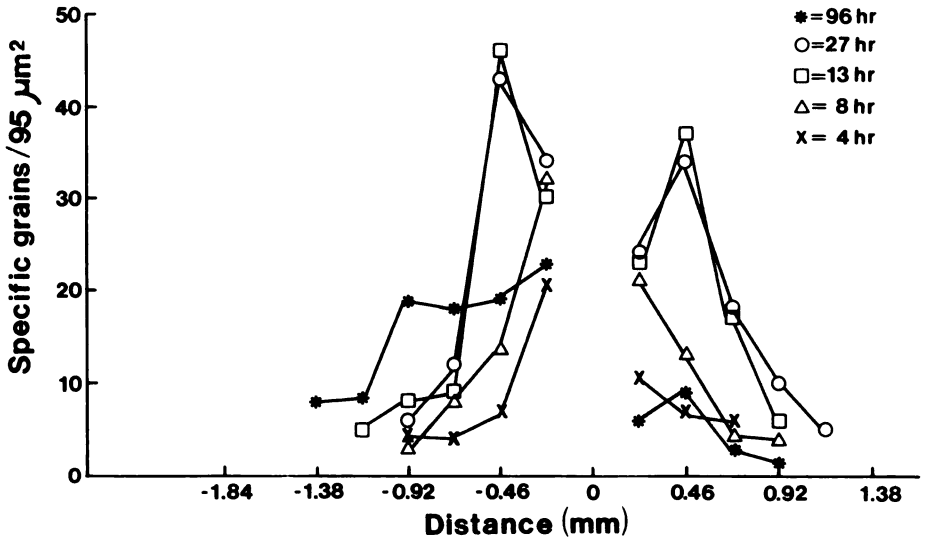

FIG. 2. *Time course of ^{125}I-CYP binding site accumulation in rat sciatic nerves*

The sciatic nerves of anesthetized rats were surgically exposed and ligated. After 4, 8, 13, 27, or 96 hr of ligation, a segment of nerve (which extended 5 mm on either side of the ligature) was removed, mounted in brain paste, and frozen in liquid nitrogen. The tissue was then sectioned and assayed for ^{125}I-CYP binding as described under Materials and Methods. Autoradiograms were generated using a 5-day exposure time. The binding data were quantified by plotting specific grain density as a function of distance along the nerve trunk. Negative *X*-coordinates correspond to points proximal to the ligature. The X-coordinate 0 is assigned to the point at which the ligature was placed. This experiment was replicated four times with similar results. Data from a typical experiment are shown.

support *beta*-adrenergic receptor transport. The accumulation proximal to the distal ligature (i.e., the ligature farthest from the spinal cord) was less than one-half the accumulation proximal to the proximal ligature. A significant buildup of receptors was detected distal to the proximal ligature. The grain density in the center of the isolated nerve trunk tended to be somewhat lower than the density along an unligated segment of nerve, but this decrement was too small to estimate reliably.

Agonist versus antagonist binding to transported beta-adrenergic receptors. The inhibition of ^{125}I-CYP binding by various agonists and antagonists was examined (Fig. 4, and Table 1). The rank order potency of the agonists was: isoproterenol > epinephrine ≫ norepineph-

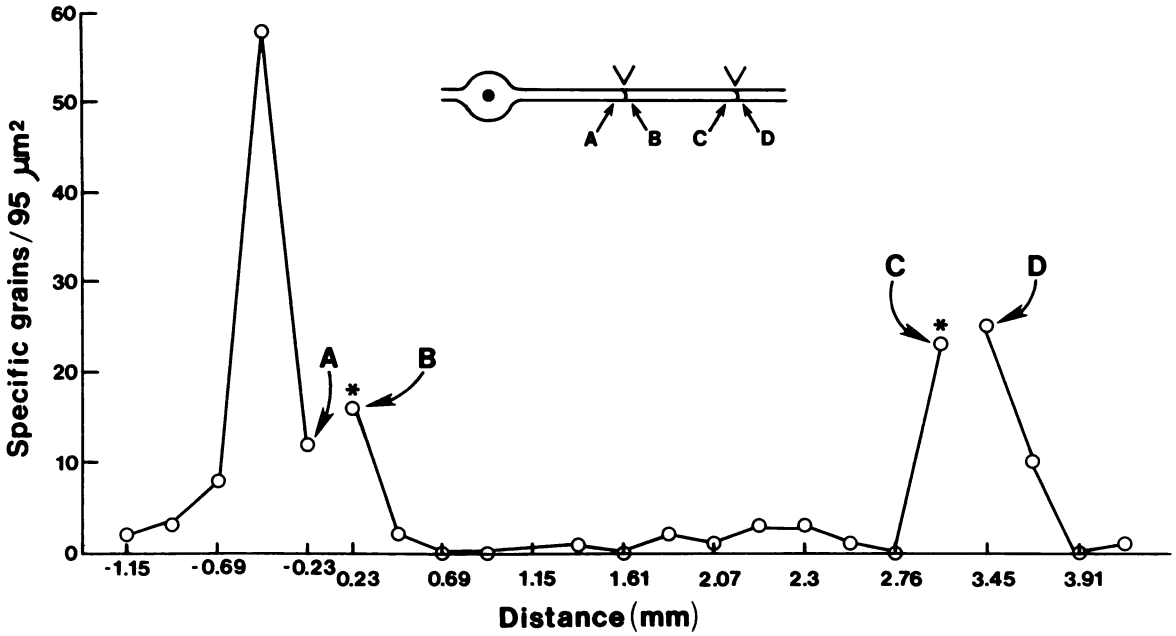

FIG. 3. *Double-ligature experiment*

Two ligatures (separated by 3–5 mm) were tied around surgically exposed rat sciatic nerves. Fifteen to eighteen hours later, a nerve segment containing the two ligatures was resected, mounted in brain paste, and frozen in liquid nitrogen. The tissue was then analyzed autoradiographically for ^{125}I-CYP binding. The experiment was repeated in four animals with similar results. Data from a typical experiment are shown. *Asterisks* indicate that the grain density of *X*-coordinates 0.23 and 2.99 is different from that at coordinates 0.46 and 2.76, respectively [$p < 0.005$ (*t*-test for two populations means)].

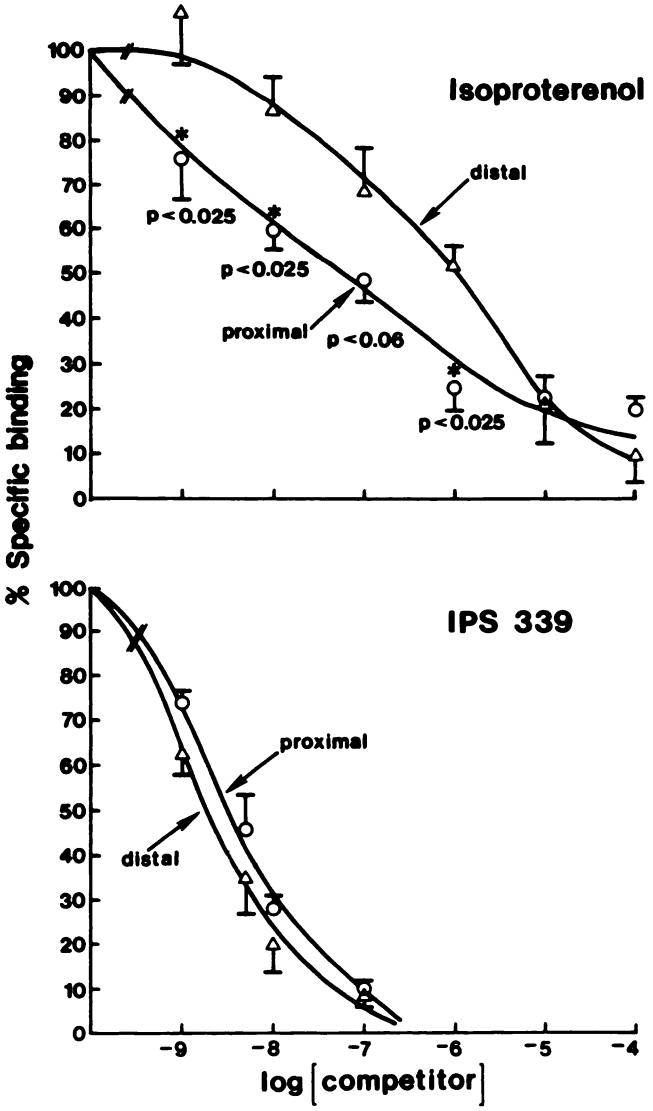

FIG. 4. *Displacement of ^{125}I-CYP binding in ligated rat sciatic nerve*

Consecutive slide-mounted frozen tissue sections (8 μm) of ligated sciatic nerves (18 hr) were incubated in 50 pM ^{125}I-CYP plus varying concentrations of displacer (isoproterenol or IPS 339) as described under Materials and Methods. Autoradiograms were then generated with a 4-day exposure time. Data are reported as means ± standard error of the mean (N = 4 for isoproterenol and N = 3 for IPS 339). *Asterisks* indicate a statistically significant difference from the corresponding value distally; the probability of the two means being the same is reported under the *asterisk*. The paired t-test was used for statistical analysis.

rine. Zinterol and IPS 339 had IC_{50} values in the nanomolar range. These data strongly indicate that at least most of the transported CYP binding sites are *beta*$_2$-adrenergic receptors. The IC_{50} values reported in Table 1 are in reasonable agreement with published values derived from the study of other tissues (20, 21).

The competition curves of agonists were different from those for antagonists. For example, isoproterenol displaced the proximal accumulation of binding sites with 20-fold greater potency than the distal accumulation of binding sites. In contrast, the antagonist IPS 339 was equipotent on both sides of the ligature. Thus, IC_{50} values for agonists were significantly (at least 10-fold) lower for the proximally accumulating receptors than for the distally accumulating receptors. The pseudo-Hill coefficients for agonists were substantially lower than those for antagonists (data not shown), as expected from the competition curves since the curves for agonists were shallower than those for antagonists.

Effect of guanine nucleotides on transported beta-adrenergic receptors. It has been shown that agonist binding to *beta*-receptors is sensitive to modulation by guanine nucleotides in amphibious (13) and mammalian tissues (14). Nucleotide sensitivity is believed to be conferred upon the receptor (R) through an interaction with a distinct guanine nucleotide-binding protein termed the N protein (N) (see ref. 16 for review).

To determine whether transported *beta*-receptors are cotransported with such a modulatory protein, the ability of GppNHp, a nonhydrolyzable GTP analogue, to inhibit isoproterenol competition was examined in tissue sections of ligated rat sciatic nerve. In preliminary experiments (Table 2), GppNHp reversed the isoproterenol inhibition of ^{125}I-CYP binding to the proximally accumulating receptors. The distally accumulating receptors appeared to be much less or not sensitive to GppNHp modulation. Also, GppNHp did not interfere with ^{125}I-CYP binding when competing agonist was not present. To characterize further the guanine nucleotide modulation, the potency of GppNHp (Fig. 5) and the pharmacology of the effect (Table 3) were examined. Proximal to the ligature, GppNHp maximally increased ^{125}I-CYP binding in the presence of 1 μM isoproterenol by 100% and exhibited an EC_{50} of 0.06 μM. Distally, GppNHp exerted a much less pronounced effect on ^{125}I-CYP binding in the presence of isoproterenol. Although the

TABLE 1

Competition for ^{125}I-CYP binding in rat sciatic nerve

Longitudinal, mounted frozen sections of ligated nerve were incubated with various concentrations of drugs and 50 pM ^{125}I-CYP. Serial sections were used to ensure a good degree of reproducibility. Grains were counted no farther than 0.23 mm from the ligature proximally and distally. The ligation time was 20 hr, and the autoradiograms were exposed for 4 days (see text for details).

The IC_{50} values refer to competition for binding no farther than 0.23 mm from the ligature on either side. Statistical analysis was performed using the t-test (two population means). Data are reported as means ± standard error of the mean; N = 3 or 4 for each determination.

Drug	IC_{50}	
	Proximal	Distal
	nM	
Agonists		
Isoproterenol	94 ± 66	1751 ± 791[a]
Epinephrine	138 ± 64	4670 ± 1790[a]
Norepinephrine	4790 ± 2290	49900 ± 38800[b]
Zinterol[c]	2.00 ± 0.68	25.0 ± 11[a]
Antagonists		
IPS 339	3.77 ± 0.41	1.99 ± 0.86
^{125}I-CYP	0.114 ± 0.023	0.0890 ± 0.017

[a] Different from proximal value ($p < 0.05$).

[b] Different from proximal value ($p < 0.15$).

[c] Zinterol is actually a partial agonist; it is included under the agonist heading for simplicity.

TABLE 2

Effect of GppNHp on isoproterenol inhibition of ^{125}I-CYP binding in rat sciatic nerve

See legend to Table 1 for details. Data are expressed as specific grains per 95 μm^2 and are reported as means ± standard error of the mean.

Addition	Proximal	Distal
None	43 ± 3	33 ± 1
GppNHp, 100 μM	39 ± 3	32 ± 2
Isoproterenol, 1 μM	14 ± 3[a]	18 ± 1
GppNHp, 100 μM + isoproterenol, 1 μM	27 ± 2	18 ± 2

[a] Different from binding in the absence of isoproterenol [$p < 0.025$ (paired t-test); $N = 4$].

changes never reached the 95% confidence level of statistical significance, there seemed to be a trend for GppNHp to reverse isoproterenol inhibition of ^{125}I-CYP binding distal to the ligature in the presence of isoproterenol. The ability of various nucleotides to substitute for GppNHp was examined (Table 3). The following rank order of potency was apparent at 10 μM: GTP = GDP > GMP > ATP. At 10 μM, GTP produced a 117% increase in ^{125}I-CYP binding whereas ATP produced only a 33% increase in the presence of isoproterenol. This pharmacological profile suggests that the GppNHp inhibition of isoproterenol binding may be mediated by the *N* protein (e.g., see refs. 13 and 16).

DISCUSSION

Beta-receptors, identified here as ^{125}I-CYP binding sites, flow in both directions in the sciatic nerve. As mentioned above, they may be utilized as physiological presynaptic receptors (1–5). The buildup at the ligatures is time-dependent, reaching a maximum at about 13 hr. One can estimate the rate of receptor transport by dividing the total accumulation of receptors in 24 hr by the density of receptors along an unligated 1-mm segment of nerve. Using this approach, the rate of anterograde *beta*-receptor transport is 18 ± 1 mm/day (mean ± SEM, n = 7) and that of retrograde transport is 13 ± 2 mm/day (mean ± SEM, n = 7) during the first 24 hr of ligation. However, these calculations underestimate the true transport rate since they assume that 100% of the receptors present in the nerve are moving at any given time, which apparently is not true (see below).

Different classes of molecules appear to be transported along axons at rates which vary from 0.5 to 300 mm/day (22). Material which is transported at a rate greater than 20–30 mm/day is said to move by axonal transport or fast transport, whereas that moving less than 20 mm/day moves by axoplasmic flow or slow transport (23). It appears that isolated axons can support fast transport, whereas continuity between the cell body and axon is required for slow transport to occur (see refs. 23 and 24 for review). Thus, the results of the double-ligature experiment suggest that *beta*-receptors move by fast rather than by slow transport since the accumulation of *beta*-receptors proximal to the second, more distal, ligature suggests that the transport apparatus is locally present and functional along the axon. The accumulation of receptors distal to the first, more proximal, ligature is presumably due to the retrograde transport of material in the isolated segment, some of which may arise from the turnaround of anterogradely transported material at the distal ligature (25). Only a slight decrease in receptor density was detected in the middle of the isolated nerve trunk. This result suggests that a relatively small fraction

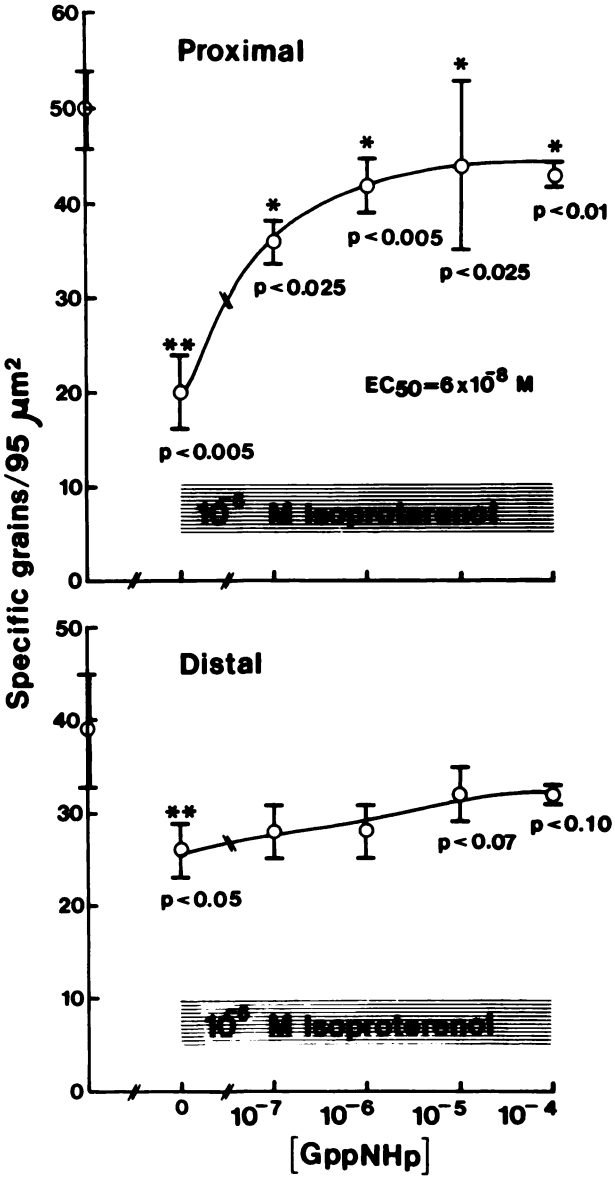

FIG. 5. *Potency of GppNHp in reversing isoproterenol inhibition of ^{125}I-CYP binding*

Consecutive slide-mounted tissue sections (8 μm) of ligated (18 hr) rat sciatic nerve were incubated in either 50 pM ^{125}I-CYP + 50 pM ICP + 10^{-6} M isoproterenol or 50 pM ^{125}I-CYP + 10^{-6} M isoproterenol + 10^{-7} to 10^{-4} M GppNHp as described under Materials and Methods. Autoradiograms of this tissue were then generated with a 5-day exposure time. Data are reported as means ± standard error of the mean (N = 4). *Single asterisks* indicate that the binding is significantly different from that in the presence of 50 pM ^{125}I-CYP + isoproterenol; the corresponding p value is written below the standard error bar. *Double asterisks* indicate that the binding in the presence of ^{125}I-CYP + 10^{-6} M isoproterenol is significantly different from binding in the presence of ^{125}I-CYP alone; the corresponding p value is written below. Statistical analysis was performed with the paired t-test.

TABLE 3

Pharmacology of GppNHp effect on beta-receptor binding in rat sciatic nerve

Data are expressed as specific grains per 95 μm^2 and are reported as means ± standard error of the mean; $N = 4$. Consecutive slide-mounted tissue sections (8 μm) of ligated sciatic nerve (18 hr) were incubated in 50 pM ^{125}I-ICP, 50 pM ^{125}I-ICP + 10^{-6} M isoproterenol, or 50 pM ^{125}I-ICP + 10^{-6} M isoproterenol + 10^{-5} M nucleotide.

Addition	Proximal	Distal
None	47 ± 6[a]	29 ± 4[b]
Isoproterenol, 1 μM	12 ± 2	17 ± 2
Isoproterenol, 1 μM + GTP, 10 μM	26 ± 4[c]	17 ± 1
Isoproterenol, 1 μM + GDP, 10 μM	24 ± 3[a]	16 ± 2
Isoproterenol, 1 μM + GMP, 10 μM	19 ± 2[a]	16 ± 1
Isoproterenol, 1 μM + ATP, 10 μM	16 ± 2[c]	15 ± 2

[a] Specific binding significantly different from that in the presence of isoproterenol alone (paired *t*-test): $p < 0.005$.

[b] See footnote *a* for comparison: $p < 0.05$.

[c] See footnote *a* for comparison: $p < 0.025$.

of the receptors present in the nerve trunk are actually moving at any given time. Thus, the clearance of receptors from the segment is very low and the precise rate of flow is difficult to determine and will require additional experiments, but it seems to be in the fast-flow catetory like that of other receptors (9, 11).

Beta-adrenergic receptors accumulated proximally and distally to ligatures at a constant rate during the first 13 hr of ligation. The decrease in the rate of accumulation at 27 hr is not understood. It may be a sequela of having injured the nerve trunk by ligation (see ref. 26 for review); if turnaround of receptors were to be established several hours after ligation, for example, an apparent decrease in the rate of accumulation would be expected. The distal loss of ^{125}I-CYP binding sites following 96 hr of ligation is probably due to axonal degeneration (e.g., see ref. 26). Thus, we interpret the loss of binding distally to mean that the bulk of the *beta*-receptors identified in this study is associated with neural elements (versus glia or inflammatory cells). The precise anatomic locus of the *beta*-receptors remains to be determined inasmuch as the class of axons in the nerve trunk which has the receptors is unknown. However, we have localized ^{125}I-CYP binding sites to cells in the superior cervical ganglion and to fibers originating from the ganglion.[6] Thus, at least some of the sciatic *beta*-receptors presumably are associated with sympathetic fibers.

The biochemical data presented in this study suggest that most of the presynaptic *beta*-adrenergic receptors in the rat sciatic nerve are $beta_2$-receptors. Thus, Zinterol and IPS 339 were potent inhibitors of ^{125}I-CYP binding, practolol was a weak inhibitor, and the rank order of agonist potency was isoproterenol > epinephrine ≫ norepinephrine. It is of interest to note that $beta_1$-receptor transport has been reported in the rat central nervous system (27). It is possible that a small fraction of receptors in sciatic nerve is also $beta_1$, although our data suggest that the bulk of the receptors is the $beta_2$ type.

Kent *et al.* (21) have shown that the $beta_1$-adrenergic receptor in frog erythrocytes exists in two states distinguished by differing affinities for agonists (viz., a high- and a low-affinity agonist binding state). Antagonists were shown to bind to both states of the receptor with the same high affinity. In the present study, agonist competition experiments have indicated that the receptors accumulating proximal to the ligature (*P* receptors) bind agonists with a high affinity. The observed IC_{50} values of isoproterenol and epinephrine at the *P* receptors are in the same range as their corresponding K_i values at the high-affinity agonist binding site in erythrocytes (21). The IC_{50} values of isoproterenol and epinephrine at the distally accumulating receptors (*D* receptors) are close to their K_i values at the low-affinity binding site (21). Thus, most of the *P* receptors and *D* receptors appear to be similar to the erythrocyte high- and low-affinity agonist receptors, respectively.

Kent *et al.* (21) have also suggested that the high-affinity agonist binding state is converted to a low-affinity state in the presence of guanine nucleotides. In other words, the high-affinity agonist binding state is a GTP-sensitive state of the receptor. In our tissue preparation, nucleotides can diffuse into the tissue and influence receptor binding (11). In the presence study, we have shown that the *P* receptors are sensitive to modulation by guanine nucleotides, whereas the *D* receptors are much less sensitive or not sensitive. This result supports the proposed analogy between *P* receptors and erythrocyte high-affinity binding sites, and *D* receptors and erythrocyte low-affinity binding sites. Although the data strongly suggest that the *P* receptors are high-affinity sites and the *D* receptors are low-affinity sites, neither population of receptors is likely to be homogeneous, since agonist competition for ^{125}I-CYP binding occurs over greater than 2 orders of magnitude and pseudo-Hill coefficients for agonists were less than those for antagonists. Thus, high- and low-affinity agonist binding sites probably accumulate on both sides of the ligature, but high-affinity sites predominate proximally and low-affinity sites predominate distally.

Possible explanations for the observed differences between *P* and *D* receptors have been discussed elsewhere (11). A reasonable explanation is that the *P* and *D* receptors differ with regard to their functional association with a modulatory protein. It is well known that the receptor molecule, or the hormone-binding protein (*R*), of the *beta*-receptor complex can interact with a distinct regulatory protein (*N*) that, *inter alia*, regulates the affinity of *R* for agonist and confers nucleotide sensitivity upon *R* (see ref. 16 for review). It is believed that, when *R* and *N* are associated, *R* binds agonists with high affinity and is sensitive to guanine nucleotide regulation. The pharmacology of the GTP effect in ligated rat sciatic nerves is consistent with the possibility that guanine nucleotides exert their effect on the transported receptors via a protein which is similar to the *N* protein (for comparison, see ref. 13) and is cotransported and functionally associated with the *beta*-receptor (*R*). The difference in the nucleotide sensitivity of *P* and *D* receptors could thus mean that the two populations differ with regard to coupling between *R* and *N*. This could result from differences in the amount or in the availability of *N* proximal and distal to the ligature, or in the functional efficacy of *R*-*N* interactions.

[6] M. A. Zarbin and M. J. Kuhar, unpublished data.

If our interpretation of the GTP effect is correct, then it seems likely that the greater part of the assembly of the *beta*-receptor complex occurs in the cell body and not at the synaptic terminal. The transported complex could be viewed as a potentially functional unit which requires incorporation into the plasmalemma in order to assume a physiologically functional role. Anterogradely transported opiate (28), cholecystokinin, *alpha*$_2$-adrenergic receptors,[7] and muscarinic receptors (11) are also sensitive to GTP modulation. In the case of *alpha*$_2$ and muscarinic receptors, the anterogradely transported binding sites are much more sensitive to GTP modulation than are the retrogradely transported sites (11),[6] and the nucleotide pharmacology of this effect suggests that it is due to an *R-N* interaction.[8] Thus, many types of neurotransmitter receptor complexes may be assembled in the soma.

If anterogradely and retrogradely transported *beta*-adrenergic receptors are examples of the *beta*-receptor at early and late stages of its life cycle, respectively, then the data presented here could mean that *beta*-receptors undergo changes in their agonist binding properties and sensitivity to regulatory substances during the sequences of anterograde transport to, insertion in, and removal from the plasma membrane. In this regard, it is of interest to note that Chuang *et al.* (29) have shown that *beta*-receptors internalized from the surface of frog erythrocytes (after densensitization) are not sensitive to GTP. In any case, the anterogradely flowing receptors have binding properties different from those moving in retrograde direction.

Preliminary reports of these findings have been presented elsewhere (30).

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