MOLECULAR PHARMACOLOGY, 16, 441-448

# Distribution and Function of Adrenergic Receptors in the Urinary Bladder of the Rabbit

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(Received February 20, 1979)
(Accepted April 20, 1979)

SUMMARY

LEVIN, R. M. AND A. J. WEIN. Distribution and function of adrenergic receptors in the urinary bladder of the rabbit. *Mol. Pharmacol.* **16**: 441-448 (1979).

Studies of the response of isolated rabbit urinary bladder strips to adrenergic agents indicate the presence of both *alpha* and *beta*-adrenergic receptors in bladder smooth muscle. Using [$^3$H]dihydroergocryptine and [$^3$H]dihydroalprenolol, we characterized the *alpha* and beta-adrenergic receptors in the base and body of the rabbit urinary bladder. The density of *alpha* receptors (in femtomoles per milligram of protein) was significantly greater in the bladder base (78 ± 10) than in the body (18 ± 4), whereas the density of *beta* receptors was significantly more concentrated in the body (96 ± 10) than in the base (42 ± 6). This uneven receptor distribution correlated well with the physiological response of isolated bladder strips to various adrenergic agonists. Methoxamine (an *alpha* agonist) stimulated contractility to a significantly greater degree in the musculature of the bladder base than in that of the body. Isoproterenol (a *beta* agonist) more effectively relaxed the bladder body than the bladder base. Epinephrine (*alpha* and *beta* agonist) produced a dose-dependent relaxation in the bladder body, whereas the bladder base responded to the same concentrations with a dose-dependent increase in contractility. We conclude from these studies that the greater response of bladder base to *alpha*-adrenergic agonists and of bladder body to *beta*-adrenergic agonists results from the predominance of *alpha* receptors in the bladder base and of *beta* receptors in the bladder body.

## INTRODUCTION

The role of the sympathetic nervous system in regulating bladder function is not completely understood. It is currently believed that the bladder contains both *alpha* and *beta* adrenergic receptors that function to increase the storage capacity of the bladder and improve continence (1, 2). Indirect support for this theory comes from physiological studies on the mechanical response of smooth muscle strips isolated from urinary bladder (of several species) to adrenergic agents (1-9). These studies indicate that strips isolated from bladder body respond to *beta*-adrenergic stimulation (relaxation) to a greater degree than strips isolated from bladder base, whereas strips from bladder base respond better to *alpha* agonists (contraction). Thus, adrenergic stimulation may be expected to relax the bladder body (*beta* effect) and contract the bladder base (*alpha* effect), both of which would facilitate urinary storage.

In order to study the adrenergic innervation of the urinary bladder directly, we adapted both *alpha* and *beta* receptor bind-

This work was supported in part by grants from the McCabe Foundation and the Veterans Administration Medical Center, Philadelphia, Pennsylvania.



0026-895X/79/050441-08$02.00/0

ing assays, using [$^3$H]DHE and [$^3$H]DHA[1] with rabbit urinary bladder. We then compared the adrenergic receptor distribution with the pharmacological response of isolated bladder strips to the adrenergic agonists methoxamine, isoproterenol, and epinephrine. The evidence presented in these studies provides direct support for the proposition that adrenergic stimulation may improve urine storage via the relaxation of the bladder dome and contraction of the bladder base.

## MATERIALS AND METHODS

*Binding assays.* The urinary bladders of 18 adult male white New Zealand rabbits were removed under light sodium pentobarbital anesthesia. After rapidly removing the serosa and mucosal layers, the tissues were used either immediately or frozen and stored under liquid nitrogen.

*Tissue preparation.* Tissue samples were rapidly weighed and homogenized in ice-cold buffer (50–100 mg/ml) using a Brinkman Polytron homogenizer. For the *alpha* adrenergic assay, the buffer was 50 mM Tris, pH 7.5, containing 10 mM $MgCl_2$; for the *beta* adrenergic assay, the buffer was 50 mM Tris, pH 8.0, containing 3 mM $MgCl_2$.

The homogenate was passed through a double layer of gauze and used immediately for the receptor binding assay.

*Beta-adrenergic receptor assay (6).* 100 μl of homogenate was incubated in the presence and absence of 10 μM propranolol and concentrations of [$^3$H]DHA varying between 0.2 and 90 nM in a total volume of 140 μl for 10 min at 37°. The reaction was stopped by the addition of 4 ml ice-cold buffer and rapidly filtered through a Whatman GFC glass fiber filter. The filter was washed with three 5 ml portions of ice-cold buffer. The filters were then placed in a scintillation vial with 5 ml of scintillation fluid and the radioactivity determined by liquid scintillation spectrometry.

Alpha-*adrenergic receptor assay* (7). One hundred microliters of homogenate were incubated in the presence and absence of 10 μM phentolamine and concentrations of $^3$H-DHE varying between 0.2 and 90 nM in a total volume of 140 μl for 15 min at 27°. The reaction was stopped with the addition of 4 ml ice-cold buffer and rapidly filtered through a Whatman GFC glass fiber filter. The filter was washed with 3–10 ml portions of ice-cold buffer. The filters were placed in scintillation vials with 5 ml scintillation fluid and the radioactivity determined.

*Calculations.* Specific binding of [$^3$H]-DHA or [$^3$H]DHE was calculated by subtracting the binding in the presence of either propranolol or phentolamine (nonspecific binding) from the total binding. The specific binding ranged between 35–55% of the total counts bound per membrane.

The specific binding was determined at each of six concentrations of [$^3$H]DHA and [$^3$H]DHE ranging between 0.2 nM and 90 nM. The dissociation constant ($K_d$) and the number of binding sites (N) was determined by Scatchard analysis (8) of the specific binding.

Protein concentrations were determined by the method of Lowry *et al.* (9).

*Materials.* [$^3$H]DHA (48.6 Ci/mMol) and [$^3$H]DHE (38.8 Ci/mMol) were obtained from New England Nuclear. Other reagents were obtained from general commercial sources.

*Muscle Bath Studies.* Urinary bladders of 18 adult, white, male New Zealand rabbits were removed under light sodium pentobarbital anesthesia. The bladders were dissected free of fat and serosa and separated between base and body at the level of the ureters. Longitudinal strips (1 cm × 0.5 cm) of bladder base and body were mounted in a 30 ml glass chamber containing Tyrodes solution (125 mM NaCl; 2.7 mM KCl; 0.4 mM $NaHPO_4$; 1.8 mM $CaCl_2$; 0.9 mM $MgCl_2$, 23.8 mM $NaHCO_3$ and 0.2% glucose) equilibrated with a gas mixture of 95% $O_2$, 5% $CO_2$ and maintained at 37°. Contractility was monitored using a Grass force displacement transducer connected to a 4 channel Beckman recorder.

After equilibrating for approximately 1 hour, a tension of 1 g was placed on each strip. Pharmacological agents were dissolved in deionized water and added in 50 μl aliquots. Dose response curves were performed by the sequential addition of drug

[1] The abbreviations used are: [$^3$H]DHE, [$^3$H]dihydroergocryptine; [$^3$H]DHA, [$^3$H]dihydroalprenolol.

(in a cumulative manner) at 5 min intervals. Drug concentrations presented are the total concentrations in the bath. A minimum of six concentrations of drug were used for each curve. Between curves, the tissues were washed four times with 30 ml fresh oxygenated buffer (at 37°) and allowed to recover for 30 min.

## RESULTS

Preliminary studies on the effect of pentobarbital anesthesia on both receptor characteristics and response of isolated bladders to adrenergic agonists (in rats) showed that there were no differences between tissues isolated from rats anesthetized with pentobarbital or rats sacrificed by decapitation.

*Binding studies.* The specific binding of both [$^3$H]DHE and [$^3$H]DHA to homogenates of bladder were found to be saturable, of high affinity, and had characteristics consistent with their identification as specific *alpha* and *beta* adrenergic receptors (6, 7). These characteristics include stereospecificity, linearity with increasing protein concentration, and rate of equilibration studies.

Figure 1 shows representative examples of the specific binding of both [$^3$H]DHE and [$^3$H]DHA to homogenates of bladder base and body. Figure 2 presents the Scatchard analysis derived from the curves presented in Figure 1. Comparison of the binding characteristics of bladder base and dome demonstrate that a significantly greater number[2] of *alpha* receptors, [$^3$H]-DHE binding sites, are present in bladder base (78 ± 10 fmoles/mg protein)[3] than are present in bladder body (18 ± 4 fmoles/mg protein.[3] The number of *beta* receptors, [$^3$H]DHA binding sites, is significantly greater[4] in bladder body (96 ± 10 fmoles/mg protein)[3] than in bladder base (42 ± 6 fmoles/mg protein).[3]

There was no difference in the dissociation constants ($K_d$) for either [$^3$H]DHE binding or [$^3$H]DHA binding between bladder base and body. For [$^3$H]DHE the $K_d$ was approximately 7 nM for both tissues; for [$^3$H]DHA, the $K_d$ was approximately 5 nM for both tissues.

Homogenates were used in these studies in order to determine the total number of receptor sites present in the tissue. Subcellular distribution studies on the specific binding of [$^3$H]DHE and [$^3$H]DHA to particulate preparations of rabbit bladder (Table 1) demonstrated that although the specific binding to the 10,000 × *g* and 100,000 × *g* fractions was greater than to homogenates when calculated per milligram protein, binding to these fractions combined represented only 40% of the total binding present in the homogenates. Recovery of binding sites in these particulate preparations was 88% for [$^3$H]DHA sites and 70% for [$^3$H]DHE sites.

*Effect of methoxamine on the rabbit urinary bladder.* Figure 3 shows the response of isolated strips of bladder body and base to the alpha-agonist methoxamine. Although the response of the bladder strips to pharmacological agents is presented as "% of maximum response" for comparative purposes, the magnitude of the response in mg tension is given in the figure legends. The maximum response (increase in tension) of strips isolated from bladder base was approximately threefold higher than the response of strips of bladder body. In addition, the $ED_{50}$ for methoxamine was significantly lower for the bladder base than for the bladder body. In similar experiments, epinephrine in the presence of 50 μM propranolol also stimulated contraction in the bladder base to a much greater degree (4-fold) than in the bladder body (data not shown).

The ability of methoxamine to displace [$^3$H]DHE from *alpha* receptors isolated from the bladder dome and base is presented in Table 2. As can be seen, the $IC_{50}$ for methoxamine was the same for both tissues.

In order to determine if this difference in response observed between bladder base and body to *alpha*-adrenergic agonists was due to the inability of the musculature of the bladder body to contract more forcefully, strips of base and body were exposed to maximally effective concentrations of bethanechol (a powerful cholinergic stimulant

[2] $p < 0.001$, Student's *t*-test (unpaired).

[3] Mean ± standard error of 8 preparations.

[4] $p < 0.01$, Student's *t*-test (unpaired).

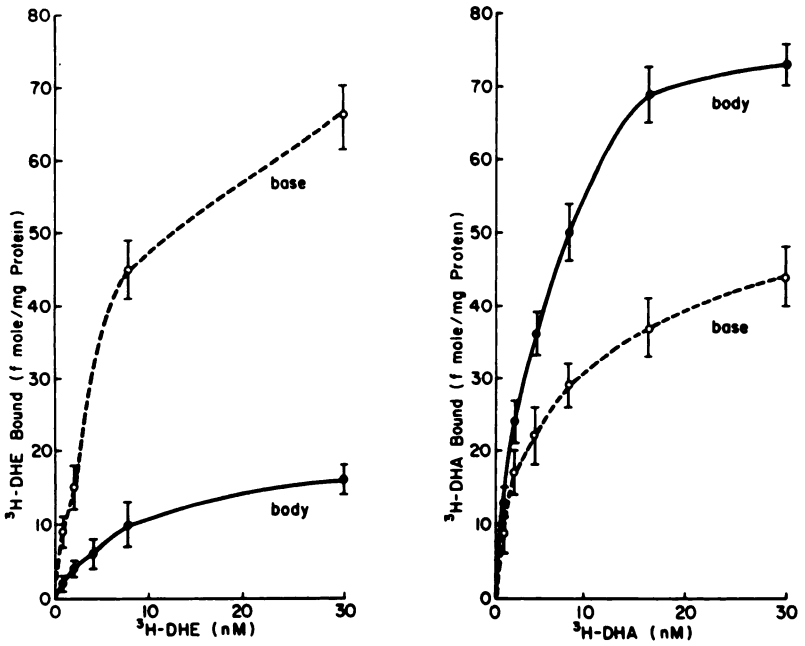


FIG. 1. *Specific binding of [$^3$H]DHE and [$^3$H]DHA to rabbit urinary bladder*

Binding experiments were performed as detailed in METHODS. The total binding was determined in the absence of phentalamine for [$^3$H]DHE binding, propranolol for [$^3$H]DHA binding, nonspecific binding was determined in the presence of 10 μM inhibitor, and specific binding was determined by subtracting nonspecific binding from total binding. Each value represents the mean of four replicates; vertical brackets indicate the standard error.

of bladder contraction). Whereas 1.5 mM methoxamine produced a 560 mg increase in tension of the bladder body, 1.5 mM bethanechol produced a 5600 mg increase in tension of the bladder body. Thus, bethanechol produced a 10-fold greater stimulation of bladder body contraction than did methoxamine.

*Effect of isoproterenol on the contractility of rabbit urinary bladder.* Figure 4 shows the response of isolated bladder strips to isoproterenol. In contrast to the effect of methoxamine, strips isolated from bladder body responded to a significantly greater degree (decrease in tension) to isoproterenol than strips isolated from bladder base. The $ED_{50}$ for isoproterenol was approximately the same for both tissues.

*Effect of epinephrine on the contractility of rabbit urinary bladder.* Figure 5 shows the effect of epinephrine on the contractility of strips isolated from bladder base and body. No adrenergic inhibitors were used in these experiments.

As can be seen, epinephrine produced a dose-dependent increase in contractility (*alpha* effect) in the bladder base while causing a dose-dependent inhibition of contractility (*beta* effect) in the bladder body.

One should note that since no inhibitors were used and the bladder base and body contain both *alpha* and *beta* receptors, the response observed in the presence of epinephrine would reflect the summation of the total receptors stimulated in the tissue. Our results indicating that both *alpha* and *beta* receptors respond to epinephrine over the same concentration range ($ED_{50}$ was approximately 4 μM for *alpha* stimulation, 1 μM for *beta* stimulation) are consistent with our radioligand displacement studies, which show that epinephrine is more effective at displacing [$^3$H]DHA from *beta* receptors than [$^3$H]DHE from *alpha* receptors (Table 2).

## DISCUSSION

Postganglionic stimulation of the sympathetic fibers innervating the urinary bladder can produce either a contractile

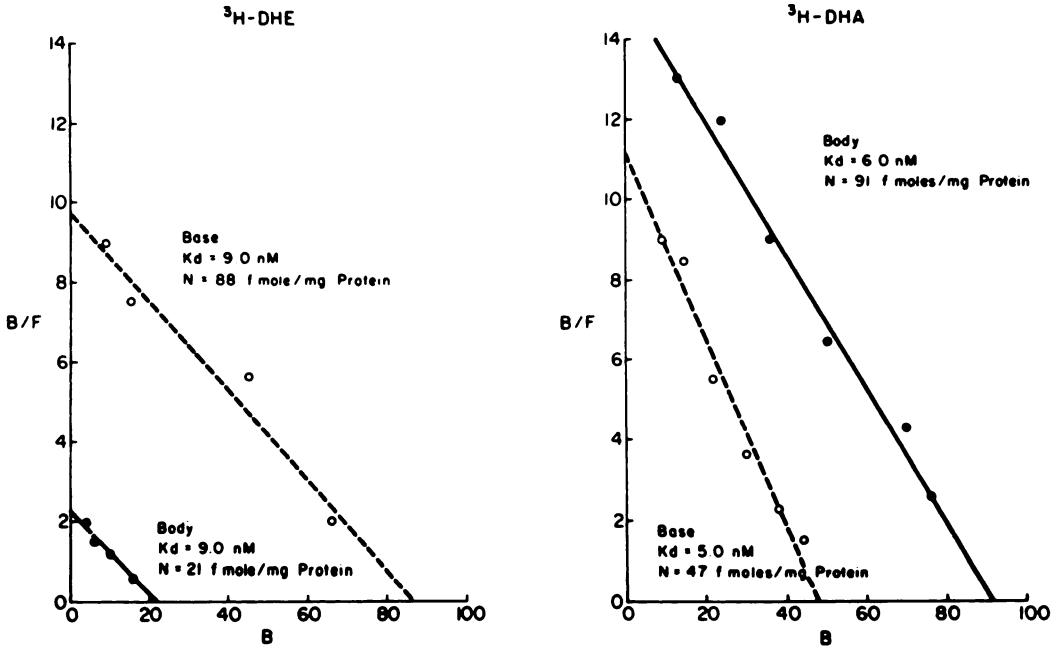


FIG. 2. *Scatchard analysis of [³H]DHE and [³H]DHA binding to rabbit urinary bladder*

The Scatchard plots of the specific binding presented in Figure 1 are shown. B = moles of [³H]DHA (or ³H-DHE) bound per mg protein; F = concentration of free ligand; $K_d$ = the dissociation constant; N = the maximum number of specific binding sites.

TABLE 1

*Subcellular distribution of [³H]DHE and [³H]DHA binding in rabbit bladder*

Each homogenate was centrifuged successively at 900 × *g* for 10 min, 10,500 × *g* for 20 min, and 100,000 × *g* for 60 min. Each particulate fraction was washed by resuspending in cold buffer and recentrifuging. The resulting washed particulate fractions were resuspended in buffer.

Binding assays were performed as described in METHODS at 20 nM [³H]DHE or 20 nM [³H]DHA in quadruplicate. The values ± standard error are the averages of four separate preparations.

| Fraction | Specific binding | | | |
|---|---|---|---|---|
| | [³H]DHE bound (base) | % of total | [³H]DHA bound (body) | % of total |
| | *(fmole/mg protein)* | | *(fmole/mg protein)* | |
| Homogenate | 63 ± 7 | 100 | 89 ± 10 | 100 |
| 900 × *g* | 65 ± 8 | 35 | 190 ± 30 | 47 |
| 10,500 × *g* | 237 ± 30 | 18 | 335 ± 50 | 20 |
| 100,000 × *g* | 142 ± 14 | 17 | 290 ± 50 | 21 |

response (*alpha* adrenergic effect), relaxation (*beta* adrenergic effect), or a biphasic response, depending on the species and techniques utilized (1, 3, 10). Physiological studies on the response of isolated bladder strips to adrenergic agents have been used to identify the presence of both *alpha* and *beta* adrenergic receptors in the bladder and indicate that both qualitative and quantitative differences may exist in adrenergic innervation of bladder base and bladder body (1, 4–6).

In order to study the adrenergic innervation directly, we have utilized receptor binding assays to characterize both *alpha* and *beta* receptors in the bladder body and base of rabbits, and compared receptor characteristics to the physiological response of isolated bladder strips to a variety of adrenergic agents.

A comparison of the adrenergic receptor density between bladder base and body demonstrates that *beta* adrenergic receptors are concentrated in bladder body whereas *alpha* receptors are concentrated in bladder base. The density of *beta* receptors in the bladder body is over five times that of *alpha* receptors in bladder body, whereas the density of *alpha* receptors in the bladder base is less than twice that of beta receptors in the bladder base. There

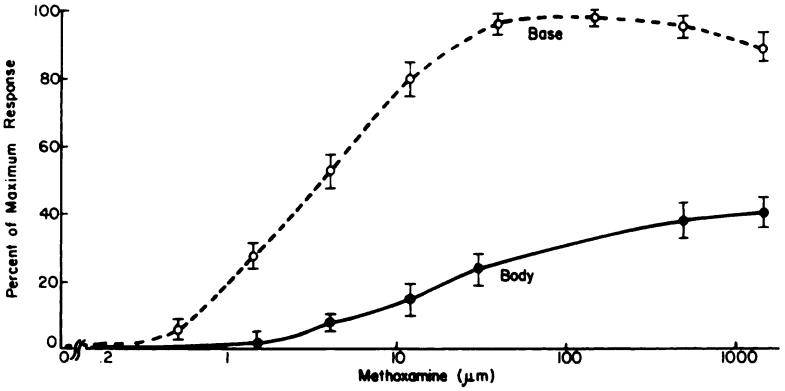


FIG. 3. *Effect of methoxamine on the rabbit urinary bladder*

The response of strips isolated from bladder base and body to methoxamine was determined as described in METHODS. The data are presented as the "% of the maximum response." 100% is equal to the maximum increase in tension of the bladder base (1600 ± 300 mg increase over basal tension). The response of the bladder dome is calculated relative to the maximal response of the base. Each value represents the mean of 6 separate preparations; vertical brackets indicate the standard error.

TABLE 2

*Displacement of [$^3$H]DHE and [$^3$H]DHA from bladder base and body by epinephrine and methoxamine*

The specific binding of [$^3$H]DHE (30 nM) and [$^3$H]DHA (30 nM) was determined in the presence and absence of seven concentrations of either epinephrine or methoxamine. The concentration required to inhibit the specific binding of the radioligand by 50% ($IC_{50}$) was determined by graphical analysis. Each value is the mean ± the standard error of 3–6 determinations.

| Agent | $IC_{50}$ | | | |
|---|---|---|---|---|
| | $^3$H-DHA | | $^3$H-DHE | |
| | Base | Body | Base | Body |
| Epinephrine | 0.6 ± 0.2 | 0.8 ± 0.2 | 1.6 ± 0.4 | 2.0 ± 0.5 |
| Methoxamine | 11 ± 2 | 13 ± 3 | — | — |

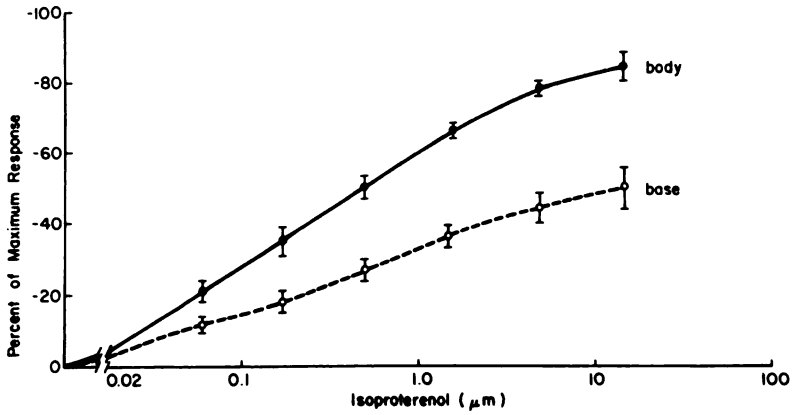


FIG. 4. *Effects of isoproterenol on rabbit urinary bladder*

The response of strips isolated from bladder base and body to isoproterenol was determined as described in METHODS. The data are presented as "% of maximum response." 100% would equal the reduction of basal tension (1000 mg) to zero. Each value represents the mean of six separate preparations; vertical brackets indicate the standard error.

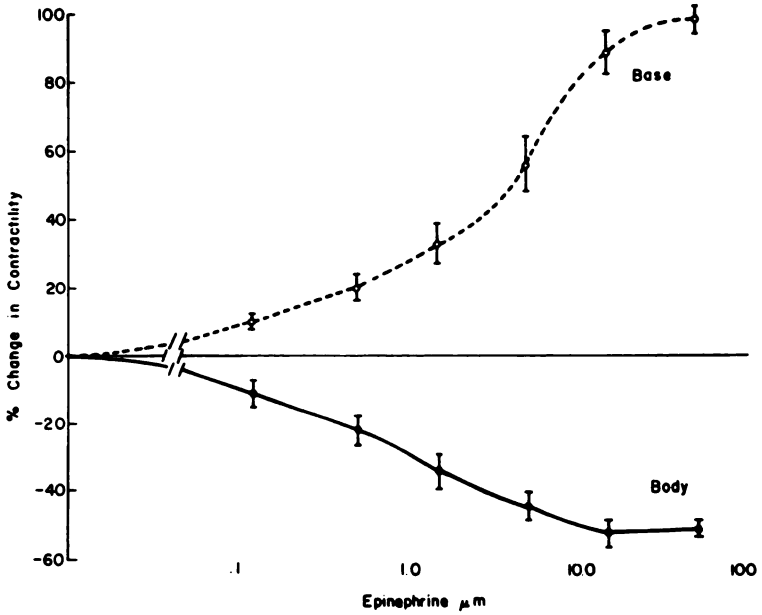


FIG. 5. *Effect of epinephrine on rabbit urinary bladder.*

The response of strips isolated from bladder base and body to epinephrine was determined as described in METHODS. The data are presented as "% change in contractility." +100% is equal to the maximum contractile response obtained in the base (1430 ± 380 mg above basal tension), −100% is equal to the reduction of basal tension (1000 mg) to zero. Each value represents the mean of seven separate preparations; vertical brackets indicate the standard error.

were no differences in the dissociation constants of adrenergic receptors isolated from bladder base and those isolated from bladder body.

The regional distribution of *alpha* and *beta* adrenergic receptors correlates well with our *in vitro* studies on the response of isolated bladder strips to adrenergic agents. Methoxamine (and epinephrine in the presence of propranolol) stimulated contraction to a significantly greater degree in the bladder base than in the bladder body. Although there was a significant difference in the $ED_{50}$ of bladder base and dome for methoxamine stimulation (similar to that demonstrated by Downie *et al.* [4]), this does not necessarily indicate a difference in the affinity of the *alpha* receptors for the agonist.

Because of the marked lower *alpha* receptor density in the bladder body as compared with the bladder base, *alpha* adrenergic stimulation of the bladder body may require that a significantly greater proportion of receptors be occupied in order to overcome the passive elastic properties of the smooth muscle and produce an observable contraction. This would be reflected as an increase in the $ED_{50}$ even though the affinity of the *alpha* receptors for methoxamine would be the same for the bladder body and base. Conversely, because of the relatively high *alpha* adrenergic density of the bladder base, maximal contractile response of the base may require proportionally less receptor-occupation than required for maximal contraction of the body. Because of the problems inherent in trying to determine receptor-ligand interaction indirectly via the measurement of contractile response, our demonstration that the affinity of *alpha* receptors isolated from the base and body for [$^3$H]DHE is equal, and that methoxamine and epinephrine are equally effective at displacing [$^3$H]DHE from *alpha* receptor preparations of the base and body, indicates that there is no qualitative difference in alpha receptors isolated from these two regions.

Isoproterenol was more effective in reducing the tension of bladder body than of musculature of bladder base. This result correlates with the predominance of *beta* receptors in the bladder body.

Epinephrine in the absence of inhibitors produced a dose-dependent increase in tension in the bladder base whereas over the same concentration range epinephrine produced a dose-dependent decrease in tension in bladder body. This response to epinephrine is similar to that reported by Salimi *et al.* (5), and would be expected from our findings that *alpha* receptors predominate in the bladder base and *beta* receptors predominate in bladder body.

We realize that the magnitude of the contractile response to autonomic agents is not solely a function of the density of receptors and that several physical factors can influence this response. In this regard, we were careful that the size of the strips from bladder base and body were equal and that they were hung in the same direction.

The following conclusions can be drawn from these studies: 1) The bladder base contains a predominance of *alpha* receptors whereas there is a predominance of *beta* receptors in the bladder body. 2) *Alpha* and *beta* receptors isolated from bladder body have characteristics identical with those isolated from bladder base. 3) The physiological response of bladder strips isolated from bladder base and body to specific adrenergic agonists is consistent with adrenergic receptor distribution.

In general, these studies support the theory that sympathetic stimulation of the urinary bladder may improve urine storage by simultaneous relaxation of the bladder body and contraction of the bladder base.

In man, there is circumstantial evidence that the sympathetic nervous system is involved in a similar way in urine storage. This evidence includes the following: 1) functional bladder outlet obstruction at the level of the bladder neck and proximal urethra has been successfully treated with *alpha* adrenergic blockade (11, 12) and 2) selected cases of detrusor hyperreactivity, enuresis, and stress urinary incontinence have been treated successfully with agents that stimulate the adrenergic receptors of the bladder and proximal urethra (13, 14).

ACKNOWLEDGMENT

We would like to thank Miss Suzan Berg and Miss Frances Shofer for their excellent technical assistance.

REFERENCES

1. Raezer, D. M., A. J. Wein, D. Jacobowitz, and J. N. Corriere, Jr. Autonomic innervation of canine urinary bladder. *Urology* **2**: 211–221, 1973.
2. Benson, G. S., D. Jacobowitz, D. M. Raezer, J. N. Corriere, Jr., and A. J. Wein. Adrenergic innervation and stimulation of canine urethra. *Urology* **7**: 337–340, 1976.
3. Edvardsen, P. and J. Seteklein. Distribution of adrenergic receptors in the urinary bladder of cats, rabbits, and guinea-pigs. *Acta. Pharmacol. et Toxicol.* 16: 437–445, 1968.
4. Downie, J. W., D. M. Dean, G. Carro-Ciampi, and S. A. Awad. A difference in sensitivity to alpha-adrenergic agonists exhibited by detrusor and bladder neck of rabbit. *Canad. J. Physiol. Pharma.* **53**: 525–530, 1975.
5. Salimi, M., J. Seteklein, and T. J. Skobba. The sensitivity of adrenergic excitatory and inhibitory receptors in the smooth muscle of the rabbit urinary bladder. *Acta Pharmacol. Toxicol.* **27**: 213–224, 1969.
6. Bylund, D. B., and S. H. Snyder. Beta adrenergic receptor binding in membrane preparations from mammalian brain. *Mol. Pharmacol.* **12**: 568–580, 1976.
7. Williams, L. T. and R. J. Lefkowitz. Molecular pharmacology of alpha adrenergic receptors: utilization of (3H) dihydroergocryptine binding in the study of pharmacological receptor alterations. *Mol. Pharmacol.* **13**: 304–313, 1977.
8. Scatchard, G. The attractions of proteins for small molecules and ions. *Ann. N. Y. Acad. Sci.* **51**: 660–672, 1949.
9. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. Protein measurement with the Folin phenol Reagent. *J. Biol. Chem.* **193**: 265, 1951.
10. Elmer, M. Stimulation of adrenergic nerve fibers to the urinary bladder of the rat. *Act. Physiol. Scand.* **94**: 517–611, 1975.
11. Khanna, O. P. Disorders of micturition: neuropharmacologic basis and results of drug therapy. *Urology* **8**: 316, 1976.
12. Krane, R. J. and C. A. Olssen. Phenoxybenzamine in neurogenic bladder dysfunction. Clinical considerations. *J. Urology* **110**: 653, 1973.
13. Mahony, D. T., R. O. Laferte, and J. E. Mahoney. Observations on sphincter augmenting effect of imipramine in children with urinary incontinence. *Urology* **2**: 317, 1973.
14. Raezer, D. M., G. S. Benson, A. J. Wein, and J. W. Duckett, Jr. The functional approach to the management of the pediatric neuropathic bladder: a clinical study. *J. Urology* **117**: 649, 1977.