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High Efficiency Coupling of *Alpha*-1 Adrenergic Receptors to Inositol Phospholipid Metabolism Revealed by Denervation of Rat Vas Deferens[1]

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ABSTRACT

The effect of surgical denervation on *alpha*-1 adrenergic receptor-stimulated inositol phosphate (IP) formation was examined in rat vas deferens. Rings of tissue from acutely reserpinized animals were incubated with [^{3}H]inositol in the presence of lithium to block IP degradation and desmethylimipramine to block neuronal uptake of norepinephrine. Eighteen days after denervation the potency of norepinephrine in stimulating [^{3}H]IP accumulation was increased 10-fold. The potency of epinephrine was increased only 3.5-fold, and the potency of phenylephrine was not altered significantly. The potency of norepinephrine in control tissues incubated with 0.1 μM desmethylimipramine was unaffected by addition of cocaine to further block neuronal uptake; however, addition of pyrogallol and pargyline to block metabolic degradation increased the potency of norepinephrine in these tissues by 6-fold. Two days after denervation there was a similar 5-fold increase in the potency of norepinephrine. In denervated tissues, the potency of norepinephrine in stimulating [^{3}H]IP accumulation was decreased about 40-fold after receptor inactivation with 1 μM phenoxybenzamine. These results suggest that there is a substantial *alpha*-1 adrenergic receptor reserve for stimulating [^{3}H]IP accumulation in rat vas deferens which is normally obscured by rapid inactivation of norepinephrine. The increase in the potency of norepinephrine after denervation appears to be due to removal of these inactivation mechanisms.

Removal of the sympathetic input to many smooth muscles increases their contractile sensitivity to norepinephrine and other agents. This increased sensitivity is due partly to loss of prejunctional uptake processes which are important in terminating the actions of norepinephrine. Often, however, there is also an enhanced postjunctional responsiveness of the smooth muscle cells to receptor activation (Fleming, 1976; Westfall, 1981). Such postjunctional supersensitivity has been variously attributed to changes in membrane potential, calcium utilization, second messenger production or cell surface receptor density or affinity (Fleming, 1976; Takenawa *et al.*, 1983).

We have studied previously the importance of changes in *alpha*-1 adrenergic receptor density after sympathetic denervation of rat vas deferens. We found that surgical denervation did not change the potency of phenylephrine in causing contractions of vas deferens, but increased the maximal contractile response (Abel *et al.*, 1985). Conversely, chronic reserpine treatment caused an increase in potency with no change in maximal response to phenylephrine (Nasseri *et al.*, 1985). Neither of these treatments affected *alpha*-1 adrenergic receptor density or affinity in vas deferens, suggesting that the changes were occurring at some event distal to the binding of agonist to the receptor. This is consistent with the observation that the supersensitivity caused by these treatments was relatively nonspecific, also being apparent with compounds acting through nonadrenergic mechanisms (Kasuya *et al.*, 1969; Abel *et al.*, 1985; Nasseri *et al.*, 1985).

Activation of *alpha*-1 adrenergic receptors in smooth muscle, as in other tissues, increases the breakdown of inositol phospholipids to inositol phosphates and diacylglycerol (Jafferji and Michell, 1976; Takenawa, 1982; Berridge and Irvine, 1984; Fox *et al.*, 1985). Because this appears to be the initial step in *alpha*-1 adrenergic receptor-mediated signal transduction, it is possible that the coupling of these receptors to IP formation might be increased by denervation. Previously, denervation has been shown to increase norepinephrine-stimulated phosphatidylinositol metabolism in a variety of tissues (Abdel-Latif *et al.*, 1978; Akhtar and Abdel-Latif, 1986; Janowsky *et al.*, 1984; Zatz, 1985; Kendall *et al.*, 1985). On the other hand, denervation of rat parotid actually interfered with the coupling of this system (Downes *et al.*, 1983), and the increased coupling in some of these tissues has been disputed (Fowler *et al.*, 1986a; Johnson *et al.*, 1987). In this manuscript we examine whether surgical denervation affects *alpha*-1 adrenergic receptor-stimulated ino-

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ABBREVIATIONS: IP, inositol phosphate; KRB, Krebs-Ringer bicarbonate buffer.

sitol phospholipid breakdown in rat vas deferens, to determine whether this mechanism could contribute to increases in postjunctional sensitivity to contractile agonists.

Methods

Drugs. The drugs used were obtained from the following sources: (−)-phenylephrine HCl, (−)-norepinephrine bitartrate, (−)-epinephrine bitartrate, pargyline HCl, crystalline reserpine and carbachol (Sigma Chemical Co., St. Louis, MO); desmethylimipramine HCl (USV Pharmaceutical Corp., Tuckahoe, NY); phenoxybenzamine HCl (Smith Kline and French Laboratories, Philadelphia, PA); pyrogallol (Fisher Scientific, Springfield, NJ); cocaine HCl (Dr. N. C. Moran, Emory University); and [^{3}H]myoinositol (16 Ci/mmol) (New England Nuclear, Boston, MA).

Surgical denervation. Male Sprague-Dawley rats (200–400 g) were anesthetized with sodium pentobarbital (35 mg/kg i.p.). One vas deferens from each animal was denervated using the method of Kasuya *et al.* (1969) as described previously (Abel *et al.*, 1985). Animals were allowed to recover and sacrificed at various time points after surgery.

Measurement of [^{3}H]IP accumulation. Animals were usually injected with reserpine (3 mg/kg i.p.) 18 to 24 hr before sacrifice on the appropriate days after surgery. Control and denervated vas deferens were removed and placed in KRB buffer containing (in millimolar) NaCl, 120; KCl, 5.5; $CaCl_2$, 2.5, NaH_2PO_4, 1.2; $MgCl_2$, 1.2; $NaHCO_3$, 20; dextrose, 11; and Ca-disodium EDTA, 0.029 preequilibrated with 95% O_2-5% CO_2. The vasa deferentia were cleaned of their serous sheath and marked off in 2 mm lengths from the prostatic end by pinching the organ transversely with a fine pair of forceps (Fox *et al.*, 1985). Approximately six to eight rings weighing 3 to 4 mg each were obtained from each vas deferens. Rings were preincubated in KRB for 30 min at 37°C. One ring was then placed in each assay tube containing 0.3 ml of KRB, 10 mM LiCl, 1 μCi of [^{3}H]inositol, 0.1 μM desmethylimipramine and drugs under study. This concentration of desmethylimipramine is sufficient to shift the dose-response curve for norepinephrine-induced contractions of vas deferens maximally to the left (Abel *et al.*, 1985). We could not use higher concentrations of desmethylimipramine, as higher concentrations antagonize competitively *alpha*-1 adrenergic receptors. [^{3}H]IP formation was determined essentially as described by Berridge *et al.* (1982). Incubations were continued for 2 hr at 37°C under 95% O_2-5% CO_2, stopped with chloroform-methanol and water soluble [^{3}H]IP's separated by anion exchange chromatography as described previously (Fox *et al.*, 1985). An aliquot (0.2 ml) of the organic phase was evaporated overnight, reconstituted with 0.3 ml of water and counted for determination of total radioactivity incorporation. Results are expressed as percentage of total incorporated radioactivity in each sample which was converted to [^{3}H]IP (% hydrolysis). Total disintegrations per minute incorporated into lipid averaged 12,588 ± 956 dpm (n = 24).

Data analysis. Dose-response curves were analyzed by linear regression of all points between 20 and 80% of the maximal response to each agonist. Unpaired Student's t tests were used to test significance, and a P values less than .05 was considered significant. Mean ± S.E.M.s are plotted in all figures. When the S.E.M. is smaller than the area of the symbol, it is not shown in the figures.

Results

Effect of denervation. Basal [^{3}H]IP accumulation in the absence of drug was reduced by about 50% in vasa deferentia denervated 18 days earlier compared to sham-operated controls (table 1). To determine whether this decrease was due to removal of endogenous agonist, animals were treated 24 hr before sacrifice with reserpine (3 mg/kg i.p.) to deplete endogenous catecholamine stores. This treatment also caused about a 50% reduction in basal [^{3}H]IP accumulation (table 1). To ensure low basal levels, all subsequent animals were pretreated with reserpine.

Dose-response curves for stimulation of [^{3}H]IP accumulation by norepinephrine were determined in vasa deferentia denervated 18 days previously and in sham-operated controls. Figure 1 shows that denervation caused a 10-fold increase in potency of norepinephrine with no change in maximum effect. Acute reserpine treatment, which had similar effects on basal levels, did not alter the potency of norepinephrine in either control or denervated animals (table 1). To determine if [^{3}H]IP accumulation was linear under the conditions studied, the time course of [^{3}H]IP accumulation in the presence of norepinephrine was determined in control and 18-day denervated tissues. [^{3}H]IP accumulation in the presence of 1 μM norepinephrine appeared to be linear for up to 2 hr in both tissues, and significant effects of denervation could be observed within 20 min (fig. 2).

It was important to determine whether the denervation-induced increase in the potency of norepinephrine also was observed with other agonists. Eighteen-day denervation caused a significant, but smaller increase in the potency of epinephrine (fig. 3). Surprisingly, denervation had no significant effect on the potency of the synthetic agonist phenylephrine (fig. 3; table 2). We also tested the response to 1 mM carbachol, which was unchanged after 18 days of denervation (data not shown).

Effect of cocaine, pargyline and pyrogallol. Inasmuch as denervation increased the potencies of the naturally occurring catecholamines but not the synthetic agonist phenylephrine, we examined the possibility that this was due to changes in catecholamine availability rather than in receptor coupling efficiency. Neuronal uptake was blocked in all experiments by 0.1 μM desmethylimipramine. To ensure that this concentration of desmethylimipramine was effectively blocking uptake and the increase in potency was not due to more effective removal of neuronal uptake in denervated tissues, we added 30 μM cocaine to control tissues to further block neuronal uptake. This treatment had no effect on the potency of norepinephrine in control preparations (table 3). However, when inhibitors of monoamine oxidase (pargyline) and catechol-O-methyltransferase (pryogallol) were also added, there was a 6-fold increase in the potency of norepinephrine (fig. 4). Therefore, inhibition

TABLE 1

Effect of acute reserpine treatment and long-term denervation on norepinephrine-stimulated [^{3}H]IP accumulation in rat vas deferens

Each value is the mean ± S.E.M. of the number of experiments indicated in parentheses.

	−log EC_{50} for Norepinephrine	Basal Hydrolysis	Maximum NE-Stimulated Hydrolysis
	M	%	%
Control (n = 5)	5.66 ± 0.124	17.4 ± 1.10	64.5 ± 0.98
Acute reserpine (n = 9)	5.62 ± 0.112	9.1 ± 1.05**	61.9 ± 1.24
18-Day denervation (n = 3)	6.67 ± 0.186**	10.4 ± 1.41**	64.0 ± 4.48
Denervation + acute reserpine (n = 3)	6.60 ± 0.052**	9.2 ± 0.92**	63.6 ± 2.21

** $P < .01$ compared to control.

of norepinephrine metabolism mimicked the effect of denervation, suggesting that such effects might be due to removal of metabolic enzymes.

Effects of short-term denervation. If the effects of denervation were caused by an impaired inactivation of catecholamines, such effects should occur very quickly. Figure 4 shows that 2 days after denervation there was a 6-fold increase in the potency of norepinephrine in this system, only slightly smaller than that observed 18 days after denervation (table 1).

Effect of receptor inactivation. The potency of norepinephrine in activating inositol phospholipid metabolism in denervated vas deferens, or in control preparations in the presence of pargyline and pyrogallol, was substantially greater than in binding to or activating the functional receptors in this tissue (Fox *et al.*, 1985; Minneman and Abel, 1984). Therefore we examined the possibility that there is an *alpha*-1 adrenergic receptor reserve for stimulating inositol phospholipid metabolism in this tissue. Pretreatment of denervated vas deferens with 0.1 μM phenoxybenzamine for 10 min caused a significant 2.8-fold shift to the right in the dose-response curve for norepinephrine-stimulated [^{3}H]IP formation without changing the maximal stimulation (fig. 5). Pretreatment with 1 μM phenoxybenzamine for 10 min caused a 40-fold shift to the right and a 50% reduction in maximum (fig. 5), consistent with the presence of a receptor reserve.

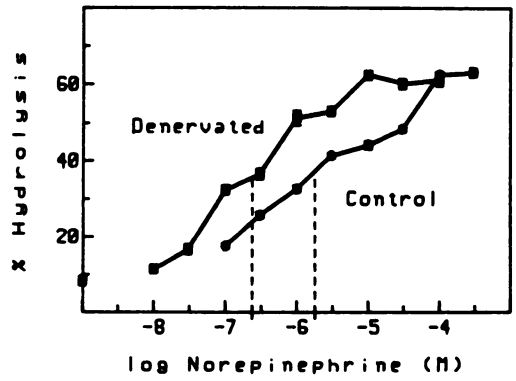

Fig. 1. Effect of 18-day denervation on norepinephrine-stimulated [^{3}H]IP accumulation in vas deferens. Desmethylimipramine (0.1 μM) was present in all tubes. Each point is the mean ± S.E.M. of duplicate determinations from three to five experiments.

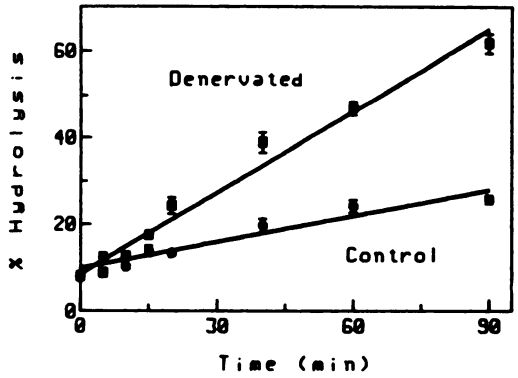

Fig. 2. Time course of [^{3}H]IP formation in control and 18-day denervated vas deferens. Desmethylimipramine (0.1 μM) and norepinephrine (1 μM) were present in all tubes for the indicated times. Each point is the mean ± S.E.M. of duplicate determinations from three experiments.

Discussion

Surgical denervation of rat vas deferens was found to increase greatly the potency of norepinephrine in stimulating [^{3}H]IP accumulation without altering the maximal degree of stimulation. Previously, denervation has been found to increase the potency of and/or maximal response to norepinephrine in stimulating [^{3}H]IP accumulation in rabbit iris (Akhtar and Abdel-Latif, 1986), hippocampus (Janowsky *et al.*, 1984) pineal (Zatz, 1985) and cerebral cortex (Kendall *et al.*, 1985; Johnson *et al.*, 1987). Such changes might be due to increases in receptor-effector coupling efficiency, possibly resulting in an increased receptor reserve (Janowsky *et al.*, 1984; Kendall *et al.*, 1985; Akhtar and Abdel-Latif, 1986). Such an effect could make an important contribution to the postsynaptic supersensitivity which becomes apparent after denervation.

However, we found that denervation caused a much smaller increase in the potency of epinephrine, and no change in potency of phenylephrine in increasing [^{3}H]IP accumulation in vas deferens. An increase in receptor-effector coupling efficiency should result in equivalent increases in potency for all agonists with a maximal intrinsic activity. Because increases were observed only for the natural catecholamine agonists norepinephrine and epinephrine and the increase was smaller for epinephrine, it was possible that changes in the availability of these compounds might be involved in the effects of denervation.

Reuptake into adrenergic nerve terminals is a major mechanism for terminating the action of norepinephrine (Iversen, 1967). Removal of this uptake system by denervation would be expected to result in large increases in the potency and/or maximal response to norepinephrine (Fleming, 1976; Westfall, 1981). In many of the previous studies examining the effect of denervation on norepinephrine stimulated [^{3}H]IP accumulation, removal of uptake processes probably played an important role in the increased responsiveness to norepinephrine which was observed, as no steps were taken to control for this "pre-

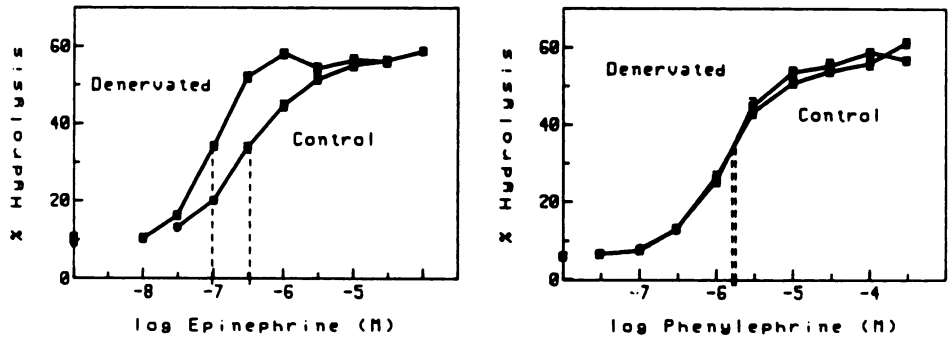

Fig. 3. Effect of 18-day denervation on epinephrine- and phenylephrine-stimulated [^{3}H] IP accumulation in vas deferens. Desmethylimipramine (0.1 μM) was present in all tubes. Each value is the mean ± S.E.M. of duplicate determinations from three experiments.

junctional" supersensitivity (Abdel-Latif *et al.*, 1978; Janowsky *et al.*, 1984; Zatz, 1985; Akhtar and Abdel-Latif, 1986). It would be difficult, therefore, to separate out increases in postjunctional receptor-effector coupling efficiency from removal of norepinephrine inactivation by reuptake. We were interested in the mechanisms of postjunctional supersensitivity, and 0.1 μM desipramine was present in all of our experiments to block norepinephrine neuronal uptake. We also found that addition of 30 μM cocaine to further block neuronal uptake had no effect on the control dose-response curve for [^{3}H]IP accumulation, suggesting that loss of the specific neuronal uptake process caused by denervation did not contribute to the increased potency of norepinephrine.

Another mechanism for terminating the actions of norepinephrine is degradation by the enzymes monoamine oxidase or catechol-O-methyltransferase. Monoamine oxidase is largely localized to the mitochondria whereas catechol-O-methyltransferase is distributed more widely. Although this inactivation mechanism is usually less important than reuptake, it gains importance when uptake is blocked. Branco *et al.* (1984) showed that surgical denervation impairs norepinephrine O-methylation and causes morphological alterations in dog saphenous vein and rabbit ear artery. Fowler *et al.* (1986b) showed that pretreatment of hippocampal slices with pargyline, an inhibitor of monoamine oxidase, potentiated the stimulation of [^{3}H]IP accumulation caused by norepinephrine, but not phenylephrine. We found that addition of pargyline as well as pyrogallol, an inhibitor of catechol-O-methyltransferase, caused a 6-fold increase in the potency of norepinephrine in stimulating [^{3}H] IP accumulation in control vas deferens. This increase was similar to that caused by 18-day denervation, raising the possibility that the effect of denervation was due to a reduction in the availability of metabolic enzymes degrading norepinephrine. This hypothesis was supported by the fact that a similar increase in the potency of norepinephrine was observed 2 days after denervation. At this time there is little or no postjunctional supersensitivity observed (Abel *et al.*, 1985), although the majority of the nerve terminals have degenerated.

These results suggest that surgical denervation does not increase the efficiency of coupling between *alpha*-1 adrenergic receptors and [^{3}H]IP accumulation in rat vas deferens. Similar results have been obtained previously in rat hippocampus (Fowler *et al.*, 1986a) and in rat cerebral cortex (Johnson *et al.*, 1987). Although our results are in apparent contradiction to studies in other tissues in which increases in receptor-effector coupling efficiency have been postulated after denervation (Janowsky *et al.*, 1984; Zatz, 1985; Akhtar and Abdel-Latif, 1986), these previous studies could not distinguish between changes in coupling efficiency and changes in norepinephrine availability caused by decreased uptake and/or metabolism (see above). On the other hand, a report by Kendall *et al.* (1985) found an increase in the intrinsic activity of phenylephrine in stimulating [^{3}H]IP accumulation in rat cerebral cortex after chemical denervation with 6-hydroxydopamine, suggesting a true increase in receptor-effector coupling efficiency. The reason for this difference is not obvious; however, our results show clearly that *alpha*-1 adrenergic receptor-stimulated inositol phospholipid metabolism in rat vas deferens is not changed by denervation. The exact relationship between contractile responses and inositol phospholipid metabolism remains to be established, but the increase in maximal contractile response caused by surgical denervation (Abel *et al.*, 1985) does not appear to be associated with receptor-stimulated inositol phospholipid metabolism.

In previous studies we found discrepancies between the apparent intrinsic efficacies of various agonists in stimulating [^{3}H]IP accumulation in rat vas deferens. Although phenylephrine and methoxamine are only partial agonists for stimulating this response in rat cerebral cortex (Minneman and Johnson, 1984; Brown *et al.*, 1984; Schoepp *et al.*, 1984), these compounds appeared to be full agonists with similar intrinsic activities to norepinephrine and epinephrine in vas deferens (Fox *et al.*, 1985). In addition, low efficacy partial agonists such as clonidine and phenylpropanolamine which do not stimulate [^{3}H]IP accumulation in brain had significant activities (40–80% of

TABLE 2

Effect of 18-day denervation on the potencies of other agonists

All animals were pretreated with reserpine (3 mg/kg). Each value is the mean ± S.E.M. of three determinations from separate experiments.

	−log EC_{50}		Antilog Ratio
	Control	18-Day denervation	
	M	*M*	
Epinephrine	6.48 ± 0.012	7.02 ± 0.028**	3.5
Phenylephrine	5.67 ± 0.089	5.80 ± 0.009	1.3

** $P < .001$ compared to control.

TABLE 3

Effect of inhibition of uptake or metabolism and short-term denervation on the potency of norepinephrine

All animals were pretreated with reserpine (3 mg/kg). All tubes contained 0.1 μM desmethylimipramine. Each value is the mean ± S.E.M. from three to nine experiments.

	−log EC_{50}	Antilog Ratio
	M	
Control	5.62 ± 0.112	1.5
+30 μM cocaine	5.80 ± 0.093	
+30 μM cocaine, +100 μM pyrogallol, +100 μM pargyline	6.41 ± 0.061**	6.2
2-Day denervation only	6.34 ± 0.280**	5.2

** $P < .01$ compared to control.

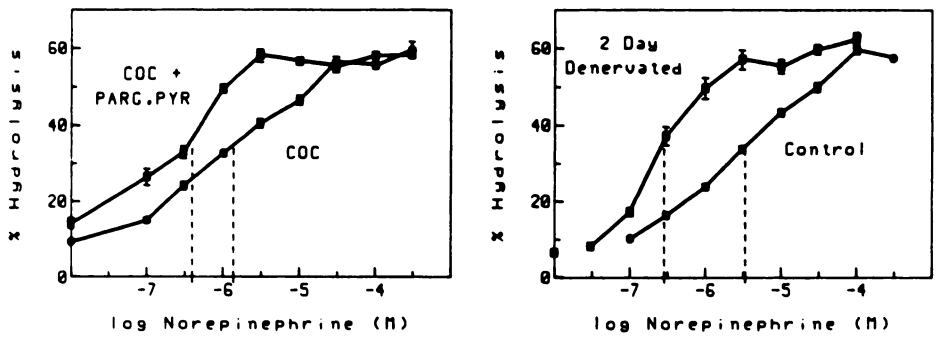

Fig. 4. Effect of cocaine, pargyline and pyrogallol and short-term denervation on norepinephrine-stimulated [^{3}H]IP accumulation in vas deferens. Desmethylimipramine (0.1 μM) was present in all tubes. Left panel, 30 μM cocaine (COC) or COC plus 0.1 mM pargyline (PARG) and 0.1 mM pyrogallol (PYR) were added. Right panel, [^{3}H]IP accumulation was measured in control and 2-day denervated tissues. Each value is the mean ± S.E.M. of duplicate determinations from three to nine experiments.

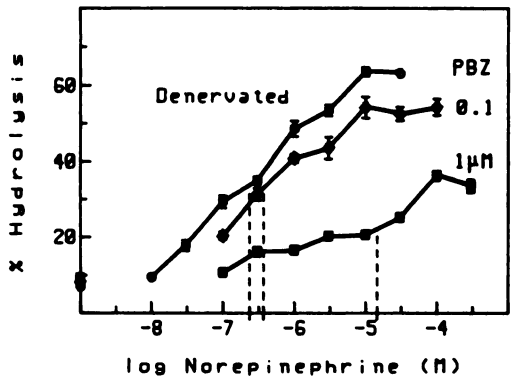

Fig. 5. Effect of phenoxybenzamine (PBZ) on norepinephrine-stimulated [^{3}H]IP accumulation in 18-day denervated vas deferens. Denervated tissues were treated with 0, 0.1 or 1.0 μM PBZ for 10 min before dose-response curves were determined. Desmethylimipramine (0.1 μM) was present in all tubes. Each value is the mean ± S.E.M. of duplicate determinations from three to eight experiments.

norepinephrine) in vas deferens. However, there appeared to be little or no receptor reserve for norepinephrine or epinephrine, as the potencies of these compounds in stimulating [^{3}H] IP accumulation in vas deferens were similar to their affinities for binding to and activating the *alpha*-1 adrenergic receptors in this tissue (Fox *et al.*, 1985; Minneman and Abel, 1984). Fox and Friedman (1987) have recently used phenoxybenzamine to obtain evidence for an *alpha*-1 adrenergic receptor reserve for stimulation of inositol phosphate production in rat vas deferens and caudal artery. Our results suggest that the potencies of norepinephrine and epinephrine in activating [^{3}H]IP accumulation in vas deferens were underestimated in our previous study, and that in the absence of catecholamine inactivation there is a significant receptor reserve for this effect. This explains why our previous results suggested that there was a receptor reserve for noncatecholamine agonists but no receptor reserve for catecholamine agonists (Fox *et al.*, 1985).

The results presented here show that the postjunctional supersensitivity caused by surgical denervation of rat vas deferens is not caused by an increase in the efficiency of coupling between *alpha*-1 adrenergic receptor stimulation and the breakdown of inositol phospholipids. These studies once again underscore the importance of controlling for inactivation pathways when the potencies of naturally occurring catecholamines are being examined. Finally, although receptor occupancy has been suggested to be linearly coupled to activation of inositol phospholipid metabolism in many tissues (Michell *et al.*, 1976; Berridge *et al.*, 1982; Fisher *et al.*, 1983; Minneman and Johnson, 1984; Brown and Brown, 1984), in rat vas deferens there is a significant *alpha*-1 adrenergic receptor reserve for stimulation of [^{3}H]IP accumulation.

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References

ABDEL-LATIF, A. A., GREEN, K., SMITH, J. P., MCPHERSON, J. C., JR. AND MATHENY, J. L.: Norepinephrine-stimulated breakdown of triphosphoinositide of rabbit iris smooth muscle: Effects of surgical sympathetic denervation and *in vivo* electrical stimulation of the sympathetic nerve of the eye. J. Neurochem. **30:** 517–525, 1978.

ABEL, P. W., JOHNSON, R. D., MARTIN, T. J. AND MINNEMAN, K. P.: Sympathetic denervation does not alter the density or properties of *alpha*-1 adrenergic receptors in rat vas deferens. J. Pharmacol. Exp. Ther. **233:** 570–577, 1985.

AKHTAR, R. A. AND ABDEL-LATIF, A. A.: Surgical sympathetic denervation increases α_1-adrenergic-mediated accumulation of myo-inositol trisphosphate and muscle contraction in rabbit iris dilator smooth muscle. J. Neurochem. **46:** 96–104, 1986.

BERRIDGE, M. J., DOWNES, C. P. AND HANLEY, M. R.: Lithium amplifies agonist-dependent phosphatidylinositol responses in brain and salivary glands. Biochem. J. **206:** 587–595, 1982.

BERRIDGE, M. J. AND IRVINE, R. F.: Inositol triphosphate, a novel second messenger in cellular signal transduction. Nature (Lond.) **312:** 325–321, 1984.

BRANCO, D., TEIXEIRA, A. A. AND OSSWALD, W.: Structural and functional alterations caused at the extraneuronal level by sympathetic denervation of blood vessels. Naunyn-Schmiedeberg's Arch. Pharmacol. **326:** 302–312, 1984.

BROWN, J. H. AND BROWN, S. L.: Agonists differentiate muscarinic receptors that inhibit cyclic AMP formation from those that stimulate phosphoinositide metabolism. J. Biol. Chem. **259:** 3777–3781, 1984.

BROWN, E., KENDALL, D. A. AND NAHORSKI, S. R.: Inositol phospholipid hydrolysis in rat cerebral cortical slices. I. Receptor characterization. J. Neurochem. **42:** 1379–1387, 1984.

DOWNES, C. P., DIBNER, M. D. AND HANLEY, M. R.: Sympathetic denervation impairs agonist-stimulated phosphatidylinositol metabolism in rat parotid glands. Biochem. J. **214:** 865–870, 1983.

FISHER, S. K., KLINGER, P. D. AND AGRANOFF, B. W.: Muscarinic agonist binding and phospholipid turnover in brain. J. Biol. Chem. **258:** 7358–7363, 1983.

FLEMING, W. W.: Variable sensitivity of excitable cells: Possible mechanisms and biological significance. *In* Reviews of Neuroscience, Vol. 2, ed. by S. Ehrenpreis and I. J. Kopin, pp. 43–90, Raven Press, New York, 1976.

FOWLER, C. J., MAGNUSSON, O., MOHAMMED, A. K., DANYSZ, W. AND ARCHER, T.: The effect of selective noradrenergic lesions upon the stimulation by noradrenaline of inositol phospholipid breakdown in rat hippocampal miniprisms. Eur. J. Pharmacol. **123:** 401–407, 1986a.

FOWLER, C. J., O'CARROLL, A.-M., COURT, J. A. AND CANDY, J. M.: Stimulation by noradrenaline of inositol phospholipid breakdown in the rat hippocampus: Effect of the ambient potassium concentration. J. Pharm. Pharmacol. **38:** 201–208, 1986b.

FOX, A. W., ABEL, P. W. AND MINNEMAN, K. P.: Activation of α_1-adrenoceptors increases ^{3}H-inositol metabolism in rat vas deferens and caudal artery. Eur. J. Pharmacol. **116:** 145–152, 1985.

FOX, A. W. AND FRIEDMAN, P. W.: Evidence for spare α_1-adrenoceptors for the accumulation of inositol phosphates in smooth muscle. J. Pharm. Pharmacol. **39:** 68–71, 1987.

IVERSEN, L. L.: The Uptake and Storage of Noradrenaline in Sympathetic Nerves, Cambridge University Press, Cambridge, 1967.

JAFFERJI, S. S. AND MICHELL, R. H.: Stimulation of phosphatidylinositol turnover by histamine, 5-hydroxytryptamine and adrenaline in the longitudinal smooth muscle of guinea pig ileum. Biochem. Pharmacol. **25:** 1429–1430, 1976.

JANOWSKY, A., LABARCA, R. AND PAUL, S. M.: Noradrenergic denervation increases α_1-adrenoreceptor-mediated inositol-phosphate accumulation in the hippocampus. Eur. J. Pharmcol. **102:** 193–194, 1984.

JOHNSON, R. D., IUVONE, P. M. AND MINNEMAN, K. P.: Regulation of *alpha*-1 adrenergic receptor density and functional responsiveness in rat brain. J. Pharmacol. Exp. Ther. **242:** 842–849, 1987.

KASUYA, Y., GOTO, K., HASHIMOTO, H., WATANABE, H., MUNAKATA, H. AND WATANABE, M.: Nonspecific denervation supersensitivity in the rat vas deferens "*in vitro*." Eur. J. Pharmacol. **8:** 177–184, 1969.

KENDALL, D. A., BROWN, E. AND NAHORSKI, S. R.: α_1-Adrenoceptor-mediated inositol phospholipid hydrolysis in rat cerebral cortex. Relationship between receptor occupancy and response and effects of denervation. Eur. J. Pharmacol. **14:** 41–52, 1985.

MICHELL, R. H., JAFFERJI, S. S. AND JONES, L. M.: Receptor occupancy dose-response curve suggests that phosphatidyl-inositol breakdown may be intrinsic to the mechanism of the muscarinic cholinergic receptor. F.E.B.S. Lett. **69:** 1–5, 1976.

MINNEMAN, K. P. AND ABEL, P. W.: Spare α_1-adrenergic receptors and the potency of agonists in rat vas deferens. Mol. Pharmacol. **25:** 56–63, 1984.

MINNEMAN, K. P. AND JOHNSON, R. D.: Characterization of *alpha*-1 adrenergic receptors linked to ^{3}H-inositol metabolism in rat cerebral cortex. J. Pharmacol. Exp. Ther. **230:** 317–323, 1984.

NASSERI, A., BARAKEH, J. F., ABEL, P. W. AND MINNEMAN, K. P.: Reserpine-induced postjunctional supersensitivity in rat vas deferens and caudal artery without changes in *alpha* adrenergic receptors. J. Pharmacol. Exp. Ther. **234:** 350–357, 1985.

SCHOEPP, D. D., KNEPPER, S. M. AND RUTLEDGE, C. O.: Norepinephrine stimulation of phosphoinositide hydrolysis in rat cerebral cortex is associated with the alpha-1-adrenoceptor. J. Neurochem. **43:** 1758–1761, 1984.

TAKENAWA, T.: Inositol phospholipids in stimulated smooth muscles. Cell Calcium **3:** 359–368, 1982.

TAKENAWA, T., MASAKI, T. AND GOTO, K.: Increase in norepinephrine-induced formation of phosphatidic acid in rat vas deferens after denervation. J. Biochem. **93:** 303–306, 1983.

WESTFALL, D. P.: Supersensitivity of smooth muscle. *In* Smooth Muscle: An Assessment of Current Knowledge, ed. by E. Bulbring, A. F. Brading, A. W. Jones and T. Tomita, pp. 285–310, Butler and Tanner, Ltd., London, 1981.

ZATZ, M.: Denervation supersensitivity of the rat pineal to norepinephrine-stimulated ^{3}H-inositide turnover revealed by lithium and a convenient procedure. J. Neurochem. **45:** 95–100, 1985.

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