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ACCELERATED COMMUNICATION

Adaptive Supersensitivity in the Guinea Pig Vas Deferens Is Associated with a Reduction in the Abundance of the α2 Subunit Isoform of Na^+/K^+-ATPase

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SUMMARY

Adaptive supersensitivity has been demonstrated previously in the guinea pig vas deferens after chronic treatment with reserpine, postganglionic denervation, or preganglionic denervation. The magnitude of the change in sensitivity was similar regardless of the method of induction; the underlying mechanism was identified as a partial depolarization secondary to reduced activity of the Na^+/K^+ pump. Experiments were conducted to quantitatively determine whether the identified losses in Na^+/K^+-ATPase activity and [^{3}H]ouabain binding were due to reductions in the levels of specific protein subunits of the sodium pump. Electrophoretic separation and quantification of the abundance of α subunit isoforms were accomplished using sodium dodecyl sulfate-polyacrylamide gel electrophoresis and slot blot analysis. Supersensitivity was induced in the guinea pig vas deferens through pretreatment with reserpine (1.0 mg/kg/day × 5 days). The abundance of the α2 subunit isoform was reduced by 41% in tissue homogenates obtained from animals treated with reserpine, compared with untreated controls. In contrast, there was no significant alteration in the α1 subunit isoform (a protein similar in size to that previously identified in vascular smooth muscle as a "truncated" form of the protein). These data suggest that the adaptation of the guinea pig vas deferens after a chronic reduction in net stimulus is mediated through a change in a specific cellular protein. This evidence supports the assignment of the α2 subunit isoform as the specific protein responsible for the development of nonspecific adaptive supersensitivity in the guinea pig vas deferens.

It is well recognized that a chronic reduction in the net stimulus received by excitable cells can lead to an adaptive increase in sensitivity of those cells to neurotransmitters and other drugs (1). In the case of smooth muscle, chronic interruption of the normal excitatory innervation routinely leads to supersensitivity not only to the excitatory transmitter but also to other unrelated excitatory agents (2). The nonspecificity of this supersensitivity suggests that the mechanism involves a cellular function that can affect responses mediated by multiple receptor systems.

In both the guinea pig vas deferens and the rabbit saphenous artery, after chronic interruption of the noradrenergic innervation (for example, by pre- or postganglionic denervation or transmitter depletion) adaptive supersensitivity develops in parallel with a partial depolarization of the smooth muscle membrane and a decline in electrogenic pumping (3-5). Extensive work with the guinea pig vas deferens, involving measurements of electrophysiology (6), Na^+/K^+-ATPase activity (4), and [^{3}H]ouabain binding (7), led to the hypothesis that the supersensitivity in the smooth muscle of the guinea pig vas deferens is ultimately the consequence of a reduction in the density of functional Na^+/K^+ pump sites (1).

The Na^+/K^+ pump is composed of several subunits. Of these, the α subunits are the ones associated with ATPase activity and the Na^+, K^+, and cardiac glycoside binding sites (8). The present work establishes, for the first time, that the guinea pig vas deferens expresses more than one isoform of the α subunit and that chronic treatment with reserpine, which depletes norepinephrine and induces adaptive supersensitivity (2), leads to a decrease in the amount of the α2 subunit protein.

Experimental Procedures

Materials. Monoclonal antibodies directed against the α1 (McK1) and α2 (McB2) subunits of the Na^+/K^+-ATPase (9, 10) were the generous gift of Dr. K. Sweadner (Harvard University). The antibodies were diluted in TBS (20 mM Tris·HCl, unbuffered, 137 mM NaCl) and stored at 4°.

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ABBREVIATION: TBS, Tris-buffered saline; TEMED, *N,N,N′,N′*-tetramethylethylenediamine; SDS, sodium dodecyl sulfate.

The antigen used for producing McK1 was the Na^+/K^+-ATPase purified from rat renal medulla (9). This antibody is specific for the $\alpha 1$ subunit of the enzyme from a number of species (8). The monoclonal antibody for the $\alpha 2$ subunit (McB2) was produced with purified rat brainstem axolemmal Na^+/K^+-ATPase (10) and has been shown to cross-react specifically with the $\alpha 2$ subunit from every mammalian species examined (8).

Pretreatment schedule and sensitivity measurements. Supersensitivity of the vas deferens was produced by chronic treatment with reserpine, according to the method described by Westfall and colleagues (11, 12). Adult male guinea pigs, (300–400 g; Hilltop Lab Animals, Inc.) were treated with reserpine (1.0 mg/kg, daily) by intraperitoneal injection. Reserpine used for injection was prepared in a vehicle composed of benzyl alcohol, citric acid, and Tween 80, as described previously (13). Injectable solutions were derived from this stock solution by appropriate dilution into distilled water. Animals pretreated with reserpine for 5 days and age-matched controls (no treatment) were euthanized by exsanguination after stunning. The dosage schedule used was similar to that previously reported to produce supersensitivity (11, 12) and to reduce catecholamines in the vas deferens to undetectable levels (14).

The development of supersensitivity was confirmed through generation of noncumulative concentration-response curves with (−)norepinephrine, as described previously by Westfall and colleagues (11, 12). Briefly, vasa deferentia were excised, desheathed, and placed in water-jacketed organ baths containing physiological salt solution, which was maintained at 37° and continually gassed with 95% O_2/5% CO_2. The composition of the physiological salt solution was as follows (in mM): NaCl, 117; KCl, 4.7; $CaCl_2$, 2.5; KH_2PO_4, 1.2; $MgSO_4$, 1.2; $NaHCO_3$, 25; and glucose, 11.5. Tissues were prepared for isometric tension recording, a resting tension of 0.2 g was applied, and the tissues were allowed to equilibrate for 1 hr before the construction of noncumulative concentration-response curves with norepinephrine. Comparisons of the sensitivity of the vas deferens between control and treated tissues were made at the level of the geometric mean EC_{50}, according to the method described by Fleming *et al.* (15).

Tissue preparation for protein fractionation and determination. Vasa deferentia were obtained as described above and stored in ice-cold protease inhibitor buffer (0.25 M sucrose, 1.0 mM EDTA, 4.0 mM phenylmethylsulfonyl fluoride, 1.0 mM 4-aminobenzamidine, 1 mg/ml bacitracin). Tissues were blotted dry, weighed, and homogenized by hand in 1.0 ml of protease inhibitor buffer/100 mg of tissue, with a Duall tissue grinder. The homogenate was microcentrifuged for 5 min to remove nonhomogenized tissue, and the supernatant was transferred to a fresh Eppendorf tube and stored at −20°. Repeated freeze-thawings of individual samples were kept to a minimum.

Protein determinations were made spectrophotometrically on tissue homogenates, according to the procedure described by Groves *et al.* (16).

SDS-polyacrylamide gel electrophoresis and semi-dry electroblotting of tissue homogenates. Electrophoresis of cellular proteins through polyacrylamide gels was accomplished with minor modifications of the conditions outlined by Ryrie and Gallagher (17). Protein samples were solubilized with sample buffer (0.005% bromphenol blue, 0.1 M dithiothreitol, 1.1% SDS, 0.25 M Tris·HCl, pH 8.9) and size fractionated in slab gels. The separating gel (12.5% acrylamide/0.125% bisacrylamide, 0.75 M Tris·HCl, pH 8.9, 0.1% SDS) was filtered through a 0.45-μm filter to remove particulates. Polymerization was initiated by the addition of ammonium persulfate (0.05%) and TEMED (0.05%). The stacking gel (6% acrylamide/0.06% bisacrylamide, 0.0624 M Tris·HCl, pH 6.7, 0.1% SDS) was similarly filtered, and ammonium persulfate (0.033%) and TEMED (0.1%) were added to the solution. The electrode buffer contained 0.025 M Tris·HCl, pH 8.6, 0.2 M glycine, and 0.1% SDS. Electrophoresis was performed under constant current conditions ranging between 15 and 20 mA.

After electrophoresis, the plates were separated and the stacking gel was removed. The separating gel was trimmed to remove excess gel and a corner was notched to designate orientation. The gel was then transferred into a tray containing OWL buffer (273 mM glycine, 37.2 mM Tris·HCl, 10% methanol), and was stored until blotted. Four pieces of 3-mm Chr chromatography paper (Whatman International Ltd.) and a piece of 0.45-μm S&S NC pure nitrocellulose (Schleicher and Schuell) were cut according to the dimensions of the gel and soaked in OWL buffer. Transfer of separated proteins was performed with a HEP1 semi-dry transfer apparatus (OWL Scientific Plastics), according to the directions supplied by the manufacturer, using a constant current of 400 mA for 2 hr. The blot was peeled from the gel, notched, and prehybridized overnight at room temperature in 2.8% nonfat dried milk in TBS/0.1% Tween.

Slot blot analysis. Two pieces of grade 470 general-purpose filter paper (Schleicher and Schuell) and a piece of 0.45-μm S&S NC pure nitrocellulose were cut according to the dimensions of the slot blot manifold (Bio-Dot SF microfiltration apparatus; Bio-Rad Laboratories) and soaked in TBS. Tissue homogenates were diluted with TBS to provide samples containing different total protein concentrations.

The nitrocellulose and blotting paper were placed within the manifold and light suction was applied. The slot blot wells were washed twice with 200 μl of TBS. After the final TBS wash, the suction was terminated and 200 μl of diluted sample were applied to the nitrocellulose in duplicate. After all the samples had been applied, the suction was reestablished and wells were washed as described previously. The blots were removed from the manifold, washed with TBS for 5 min followed by a wash with TBS/Tween for 30 min, and incubated overnight as described above.

Hybridization. After overnight prehybridization, blots were washed twice (15 min each) with TBS/Tween. The blots were then incubated for 60 min at room temperature with primary antibody (25 ml/blot). After this treatment, blots were washed three times with TBS/Tween (5 min each) and then incubated with horseradish peroxidase-conjugated goat anti-mouse secondary antibody (Bio-Rad Laboratories) in a manner similar to that described for the primary antibody. Finally, blots were washed as described above.

Amersham's enhanced chemiluminescence system was used to detect the immobilized antigens complexed with either McK1 or McB2 and horseradish peroxidase-labeled antibodies. The protocol supplied by the manufacturer was followed. Finally, the blots were wrapped in plastic wrap and placed in cassettes with X-ray film (X-Omat AR; Kodak). Exposure times varied from 15 sec to 10 min.

Densitometric measurement and statistical analysis. Densitometric analysis was performed on the slot blot X-ray film records by using the Bioscan Optimas imaging system software. The densitometer reported a real value, extracted from area screen objects, giving the mean gray value of pixels within the area boundary. Densitometric analyses were conducted on films from slot blots in which tissue homogenates from at least one control and one treated animal had been applied. This procedure ensured equivalent treatment of control and treated samples for normalization.

Slot blot replicate gray values were averaged and the mean value was plotted against the amount of protein loaded. Values obtained from the linear portion of both the experimental and control curves were normalized to the amount of total protein. The ratio of the normalized experimental to control values was multiplied by 100 and defined as the percentage of control.

Comparisons of the averaged mean percentage of control values for samples obtained from control and those from animals pretreated with reserpine were made using Student's *t* test for unpaired samples. Values were considered significantly different at $p \leq 0.05$.

Results

Concentration-response curves (data not shown) established that the pretreatment with reserpine induced a significant supersensitivity of the vas deferens to norepinephrine. The

increase in sensitivity was approximately 3-fold, similar to that reported by this laboratory in the past (12).

The guinea pig vas deferens expresses an $\alpha 2$ isoform subunit of the sodium pump, as revealed by the positive reaction with the monoclonal antibody directed against the $\alpha 2$ isoform expressed by the rat brainstem (Fig. 1, *lanes V*). As predicted from the literature, the guinea pig kidney (Fig. 1, *lane N*) does not express the $\alpha 2$ isoform, as indicated by the lack of any reaction with the antibody, whereas rat brainstem homogenates do display a positive reaction (Fig. 1, *lane B*).

As illustrated in Fig. 2, using slot blot analysis it is possible to quantitatively compare the relative tissue contents of the $\alpha 2$ isoform in homogenates of vasa deferentia from paired animals. One such slot blot is presented in Fig. 2A, to indicate the manner in which tissue samples were diluted and the degree of difference in reaction product obtained. The calculated estimate of density of reaction product (area light gray value) is linear over a large range of total protein concentrations (Fig. 2B). By comparing product density as a function of total protein in the sample, it is possible to quantitatively compare the abundance of the sodium pump $\alpha 2$ isoform in tissues from controls and from animals treated with reserpine. Tissue samples can be normalized for a given slot blot by defining the reaction product obtained in the "paired" control as 100%.

Previous studies from our laboratory (18) have also identified, in the guinea pig vas deferens, an $\alpha 1$ isoform that migrates with relatively high mobility (55–60 kDa). The brainstem and kidney demonstrated only the native full length form (112 kDa).

Comparative quantification of the abundance of the $\alpha 2$ isoform revealed an approximately 41% reduction in abundance of the subunit in tissue homogenates from animals chronically treated with reserpine, compared with those from control animals (Table 1). In contrast, in similarly determined comparisons the relative concentration of the $\alpha 1$ isoform was not significantly altered between the two groups (Table 1).

Discussion

Several important observations regarding the composition of the sodium pump in the smooth muscle of the guinea pig vas deferens are illustrated by these results. First, the guinea pig vas deferens expresses an $\alpha 2$ isoform subunit of the sodium pump, as revealed by the positive reaction with the monoclonal antibody (McB2) directed against the $\alpha 2$ isoform expressed by

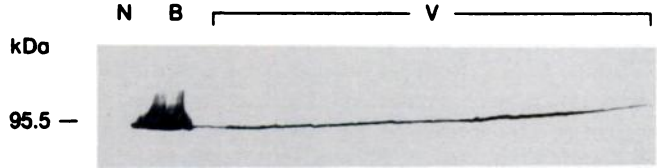

Fig. 1. The vas deferens expresses an $\alpha 2$ subunit isoform. Crude tissue homogenates were prepared from guinea pig kidney (*lane N*), rat brainstem (*lane B*), and guinea pig vas deferens (*lanes V*). Thirty microliters of total protein were size fractionated on a 12.5% SDS-polyacrylamide gel, transferred to nitrocellulose, and exposed to an anti-$\alpha 2$ antibody (McB2). Bands were visualized using a goat anti-mouse IgG conjugated to horseradish peroxidase in connection with Amersham's enhanced chemiluminescence detection system. Amersham's Rainbow markers were included in the gel and were used to determine the relative molecular masses of the proteins. Note the similarity in migration of the protein between the rat brainstem (*lane B*) and the guinea pig vas deferens (*lanes V*).

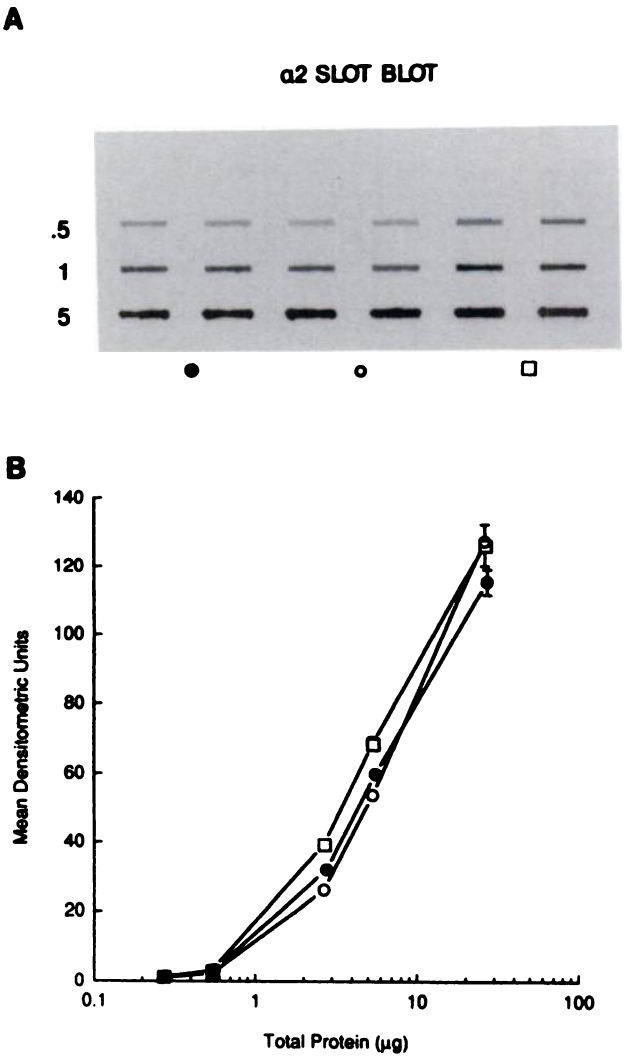

Fig. 2. Quantitative analysis of $\alpha 2$ subunit protein levels. A, Slot blot of tissue homogenates from control (□) and reserpine-pretreated animals (●, ○). Samples were diluted and loaded in duplicate as described in Experimental Procedures. Blots were exposed to an anti-$\alpha 2$ antibody (McB2) and visualized as described in the legend to Fig. 1. *Numbers*, volume (μl) of crude homogenate loaded into each well. B, Graphic representation of the relationship between protein concentration and signal production (area light gray value). Each *point* represents the average of the replicate gray values for a given protein concentration and were obtained from the slot blot illustrated in A. Note the consistency among replicates and the linear relationship between light intensity and protein concentration, as well as the reduced intensity in homogenates from treated animals, compared with control.

TABLE 1

Comparison of the $\alpha 1$ subunit and $\alpha 2$ subunit protein levels in control versus supersensitive guinea pig vasa deferentia

Slot blot replicate gray values were initially averaged. The mean value was plotted against the amount of protein loaded. Representative points from the linear parts of both the experimental and control curves were normalized to the amount of protein. The mean ratio (× 100) of the normalized experimental to control values is the mean percentage of control.

	$\alpha 1$	$\alpha 2$
Mean % of control (95% CI)[a]	115.3 (87.3–143.2)	58.6 (40.4–77.4)[b]
n[c]	12	7

[a] CI, confidence interval.
[b] Unpaired t test, $p < 0.001$.
[c] n, number of experiments.

the rat brainstem. Second, as predicted from the literature (8), the guinea pig kidney does not express an $\alpha 2$ isoform, as indicated by the lack of any reaction with the antibody. The specificity of the antibody for the $\alpha 2$ isoform is reinforced by the lack of reactivity with homogenates derived from the kidney, which does not express that protein. The lack of reaction product is not indicative of a lack of sodium pump protein, because use of a probe specific for the $\alpha 1$ isozyme (McK1) provided good reactivity with the guinea pig kidney homogenates. Third, the rat brainstem homogenates displayed a positive reaction to the antibody as well. The rat brain is an area known to express sodium pumps composed at least of both $\alpha 1$ and $\alpha 2$ isoforms [see review by Sweadner (8)]. It is also important that the size fractionation of the homogenates by electrophoresis yielded estimated $\alpha 2$ isoform molecular mass values that were nearly identical between the rat brainstem and the guinea pig vas deferens.

It is well established that the assembly of sodium pumps may utilize different isoforms of the α subunit (8, 19). Furthermore, the distribution of pump sites composed of different subunits varies among tissues and species, and the same tissue may express sodium pump sites (i.e., Na^+/K^+-ATPase enzymes) assembled with different α subunit isoforms (8). The studies reported here indicate that the guinea pig vas deferens expresses sodium pump sites that contain both $\alpha 1$ and $\alpha 2$ subunit isoforms. In addition, evaluation of the α subunit isoforms present in the guinea pig vas deferens has identified an $\alpha 1$ subunit isoform that migrates with greater mobility and closely parallels the truncated (55–60-kDa) form of the α subunit ($\alpha 1$-T), which has, until now, been identified only in vascular smooth muscle (20).

Differentiation among the isoforms of the α subunit that have been identified by cellular and molecular biological techniques can be made also by their affinity for sodium or cardiac glycosides [see review by Sweadner (8)]. Of particular importance to this study is the implication that, in rodents, the $\alpha 2$ isoform apparently possesses the highest affinity (≤ 1 μM) for cardiac glycosides, whereas the $\alpha 1$ isoform possesses lower affinity (≥ 50–60 μM) for cardiac glycosides (21–23). In addition, Brodsky (24) has shown that the high affinity isoform is the subunit that is sensitive to hormonal activation. Because previous studies have characterized the IC_{50} of ouabain in inhibiting Na^+/K^+-ATPase activity (4) and the [^{3}H]ouabain binding affinity (7) in the vas deferens to be in the <1 μM range, it is likely that the $\alpha 2$ isoform is responsible for the ouabain-sensitive sodium pump activity in this tissue. The lower molecular mass form of the $\alpha 1$ subunit (which predominates in the guinea pig vas deferens) has not been characterized with respect to its affinity for Na^+ or [^{3}H]ouabain or its physiological function. Until the characteristics of this subunit isoform are defined, any description of a physiological role for this isozyme would only be speculation.

Comparative quantification of $\alpha 2$ isoform levels revealed a 41% reduction in abundance of that subunit in tissue homogenates from animals chronically treated with reserpine, compared with those from control animals. There is no such change in the abundance of the isoform that is similar to the truncated $\alpha 1$ in these same tissue samples. A similar modulation of one specific subunit in tissues that express sodium pump units composed of the different isoforms has been observed in rat forebrain (25). Data obtained by those authors suggest that the $\alpha 2$ isoform is the subunit that is inducible in response to environmental changes. Mata *et al.* (26) quantified mRNA for $\alpha 1$ subunits in rat hypothalamus (supraoptic and paraventricular nuclei) and found a selective increase in $\alpha 1$ isoform mRNA in response to chronic depolarization. McDonough *et al.* (19, 27) have also observed modulation of both $\alpha 1$ and β protein content and mRNA abundance in rat kidney in response to alterations in the thyroid state of the animals. Thus, substantial precedent exists to support the idea that changes in the environment of a cell may elicit alterations in the expression of specific subunit proteins of the sodium pump.

A particularly striking observation from these studies is the close similarity between the magnitude of the reduction in $\alpha 2$ isoform abundance and previously reported reductions in [^{3}H] ouabain binding (7) and enzyme activity (4) in the supersensitive guinea pig vas deferens. The latter authors reported a 36% reduction in Na^+/K^+-ATPase activity after chronic treatment (1 mg/kg day $\times$ 5 days) with reserpine, whereas Wong *et al.* (7) observed a 34% reduction in the number of [^{3}H]ouabain binding sites, with no change in affinity, after a similar pretreatment schedule. The 41% reduction in protein abundance observed in this study is very similar and indicates that the loss of enzymatic activity and cardiac glycoside binding results from a reduction in the amount of the protein subunit responsible for both of these activities. Fleming and Westfall (1) have identified a reduction of electrogenic sodium pumping as the major factor contributing to the partially depolarized state of smooth muscle cells of the supersensitive guinea pig vas deferens. It is this depolarization that contributes most notably to the development of adaptive supersensitivity in this tissue. The data provided in this study indicate that the reduction in electrogenic sodium pumping is likely related to a decrease in the abundance of the $\alpha 2$ subunit isoform. Because the supersensitivity produced by chronic treatment with reserpine is identical to that produced by either preganglionic denervation (4, 11) or postganglionic denervation (4), similar magnitudes of loss of $\alpha 2$ isoform would be predicted after any treatment that leads to supersensitivity. Gerthoffer *et al.* (4) and Wong *et al.* (7) reported similar magnitudes of reduction in both enzyme activity and cardiac glycoside binding after any of several different procedures that led to the development of adaptive supersensitivity. Preliminary results from this laboratory indicate that the decline in the $\alpha 2$ isoform follows the same time course as the supersensitivity, depolarization, and decline in enzyme activity and ouabain binding (28).

Adaptational adjustments in cellular sensitivity are believed to be a homeostatic mechanism by which many excitable cells can adjust to long term (days/weeks) changes in net stimulus received (1). The data reported in this study are consistent with the general hypothesis that the cellular mechanism underlying any adaptational change in sensitivity involves cellular processing of proteins. The particular characteristics of the change in sensitivity are dictated by which proteins are modified by the cell. For example, supersensitivity in rat skeletal muscle is highly specific for nicotinic cholinomimetics and is due primarily to up-regulation of nicotinic receptors [see review by Fleming (29)]. Supersensitive atrial muscle from guinea pigs, on the other hand, demonstrates enhanced responses only to agonists that utilize the adenylyl cyclase cascade as a second messenger system, suggesting that the underlying mechanism is a change in adenylyl cyclase (30, 31). In contrast, the super-

sensitivity observed in the guinea pig vas deferens after either chronic reserpine treatment or postganglionic denervation is very nonspecific in nature and not accompanied by a change in the affinity or density of α_1-adrenoceptors (32). This nonspecific elevation in sensitivity that occurs after chronic reductions in net stimulus in the guinea pig vas deferens results from an attenuation of the electrogenic Na^+/K^+ pumping of the cell (1), which, as indicated by this study, is consequent to a reduction in the amount of the $\alpha 2$ isoforms of enzyme. The α subunit is the catalytic portion of the enzyme that contains the cardiac glycoside and Na^+ binding sites as well as ATPase activity [see review by Sweadner (8)].

Further research will be needed to determine the mechanisms by which the smooth muscle cells of the vas deferens lower the density of the $\alpha 2$ isoform of the sodium pump. Several possibilities exist, including transcriptional, translational, and post-translational modification. Modulations of both protein and mRNA of pump subunits, induced by changes in thyroid function, have been reported (19). Studies are currently under way to investigate the abundance of mRNA for the various α isoforms, to determine which step in protein processing might be altered to produce the observed reduction in the $\alpha 2$ isoform in smooth muscle of supersensitive guinea pig vas deferens.

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References

1. Fleming, W. W., and D. P. Westfall. Adaptive supersensitivity. *Handb. Exp. Pharmacol.* **90**:509–559 (1988).
2. Fleming, W. W., J. J. McPhillips, and D. P. Westfall. Postjunctional supersensitivity and subsensitivity of excitable tissues to drugs. *Ergeb. Physiol.* **68**:55–119 (1973).
3. Fleming, W. W., and D. P. Westfall. Altered resting membrane potential in the supersensitive vas deferens of the guinea pig. *J. Pharmacol. Exp. Ther.* **192**:381–389 (1975).
4. Gerthoffer, W. T., J. S. Fedan, D. P. Westfall, K. Goto, and W. W. Fleming. Involvement of the sodium-potassium pump in the mechanism of postjunctional supersensitivity of the vas deferens of the guinea pig. *J. Pharmacol. Exp. Ther.* **210**:27–36, (1979).
5. Abel, P. W., P. R. Urquilla, K. Goto, D. P. Westfall, R. L. Robinson, and W. W. Fleming. Chronic reserpine treatment alters the sensitivity and membrane potential of the rabbit saphenous artery. *J. Pharmacol. Exp. Ther.* **217**:430–439 (1981).
6. Urquilla, P. R., D. P. Westfall, K. Goto, and W. W. Fleming. The effects of ouabain and alterations in potassium concentration on the sensitivity to drugs and the membrane potential of the smooth muscle of the guinea pig and rat vas deferens. *J. Pharmacol. Exp. Ther.* **207**:347–355 (1978).
7. Wong, S. K., D. P. Westfall, J. S. Fedan, and W. W. Fleming. The involvement of the sodium potassium pump in postjunctional supersensitivity of the guinea-pig vas deferens as assessed by ^{3}H-ouabain binding. *J. Pharmacol. Exp. Ther.* **219**:163–169 (1981).
8. Sweadner, K. J. Isozymes of the Na^+/K^+-ATPase. *Biochim. Biophys. Acta* **988**:185–220 (1989).
9. Felsenfeld, D. P., and K. J. Sweadner. Fine specificity mapping and topography of an isozyme-specific epitope of the Na,K-ATPase catalytic subunit. *J. Biol. Chem.* **263**:10932–10942 (1988).
10. Urayama, O., H. Shutt, and K. J. Sweadner. Identification of three isozyme proteins of the catalytic subunit of the Na,K-ATPase in rat brain. *J. Biol. Chem.* **264**:8271–8280 (1989).
11. Westfall, D. P. Nonspecific supersensitivity of the guinea-pig vas deferens produced by decentralization and reserpine treatment. *Br. J. Pharmacol.* **39**:110–120 (1970).
12. Westfall, D. P., D. C. McClure, and W. W. Fleming. The effect of denervation, decentralization and cocaine on the response of the smooth muscle of the guinea-pig vas deferens to various drugs. *J. Pharmacol. Exp. Ther.* **181**:328–338 (1972).
13. Leyden, A. F., E. Pomerantz, and E. F. Bouchard. Pharmaceutical aspects of reserpine. *J. Am. Pharm. Assoc.* **45**:771–775 (1956).
14. Sjostrand, N. O. Effect of reserpine and hypogastric denervation on the noradrenaline content of the vas deferens and seminal vesicle of the guinea pig. *Acta. Physiol. Scand.* **56**:376–380 (1962).
15. Fleming, W. W., D. P. Westfall, I. S. de la Lande, and L. B. Jellet. Log-normal distribution of the equieffective doses of norepinephrine and acetylcholine in several tissues. *J. Pharmacol. Exp. Ther.* **181**:339–345 (1972).
16. Groves, W. E., F. C. Davis, Jr., and B. H. Sells. Spectrophotometric determination of microgram quantities of protein without nucleic acid interference. *Anal. Biochem.* **22**:195–210 (1968).
17. Ryrie, I. J., and A. Gallagher. The yeast mitochondrial ATPase complex: subunit composition and evidence for a latent protease contaminant. *Biochim. Biophys. Acta.* **545**:1–14 (1979).
18. Hershman, K. M., D. A. Taylor, and W. W. Fleming. Identification of Na^+/K^+ pump *alpha* subunits in the guinea pig vas deferens. *FASEB J.* **5**:A1190 (1992).
19. McDonough, A. A., K. Geering, and R. A. Farley. The sodium pump needs its β subunit. *FASEB J.* **4**:1598–1605 (1990).
20. Medford, R. M., R. Hyman, M. Ahmad, J. C. Allen, T. A. Pressley, P. D. Allen, and B. Nadal-Ginard. Vascular smooth muscle expresses a truncated Na+,K+-ATPase α-1 subunit isoform. *J. Biol. Chem.* **266**:18308–18312 (1991).
21. Sweadner, K. J. Enzymatic properties of separated isozymes of the Na, K-ATPase: substrate affinities, kinetic cooperativity, and ion transport stoichiometry. *J. Biol. Chem.* **260**:11508–11513 (1985).
22. Urayama, O., and K. J. Sweadner. Ouabain sensitivity of the *alpha* 3 isozyme of rat Na,K-ATPase. *Biochem. Biophys. Res. Commun.* **156**:796–800 (1988).
23. Feige, G., T. Leutert, and A. DePover. Na,K-ATPase isozymes in rat tissues: differential sensitivities to sodium, vanadate and dihydroouabain. *Prog. Clin. Biol. Res.* **268A**:377–384 (1989).
24. Brodsky, J. L. Insulin activation of brain Na^+-K^+-ATPase is mediated by α_2-form of enzyme. *Am. J. Physiol.* **258**:C812–C817 (1990).
25. Brodsky, J. L., and G. Guidotti. Sodium affinity of brain Na^+-K^+-ATPase is dependent on isozyme and environment of the pump. *Am. J. Physiol.* **258**:C803–C811 (1990).
26. Mata, M., V. Hieber, M. Beaty, M. Clevenger, and D. J. Fink. Activity-dependent regulation of Na^+,K^+-ATPase α-isoform mRNA expression *in vivo*. *J. Neurochem.* **59**:622–626 (1992).
27. McDonough, A. A., T. A. Brown, B. Horowitz, R. Chiu, J. Schlotterbeck, J. Bowen, and C. A. Schmitt. Thyroid hormone coordinately regulates Na,K-ATPase *alpha* and *beta* subunit mRNA levels in kidney. *Am. J. Physiol.* **254**:C323–C329 (1988).
28. Hershman, K. M., D. A. Taylor, and W. W. Fleming. Time dependent changes in guinea-pig vas deferens Na^+/K^+ ATPase $\alpha 2$ subunit protein levels associated with chronic reserpine treatment. *FASEB J.*, **7**:A29 (1993).
29. Fleming, W. W. Variable sensitivity of excitable cells: possible mechanisms and biological significance. *Rev. Neurosci.* **2**:43–90 (1976).
30. Fleming, W. W. Mechanisms of adaptive supersensitivity in smooth muscles vs. cardiac muscle: a brief review. *Clin. Exp. Pharmacol. Physiol.* **16**:447–450 (1989).
31. Fleming, W. W., and D. A. Taylor. Cyclic AMP and adaptive supersensitivity in guinea-pig atria. *J. Neural Transm.* **34**(suppl.):179–185 (1991).
32. Cowan, F. F., S. K. Wong, D. P. Westfall, and W. W. Fleming. Effect of postganglionic denervation and pretreatment with reserpine on *alpha*-adrenoceptors of the guinea-pig vas deferens. *Pharmacology* **30**: 289–295 (1985).

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