Changes in the Sensitivity of the External Iliac Artery to Specific Vasoconstrictors and Vasodilators Following Chronic Treatment with Reserpine, a Potent Antihypertensive Agent

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TABLE of CONTENTS

ABSTRACT

This project is designed to define the changes in sensitivity and responsiveness that occur in blood vessels (an external iliac artery) after the normal sympathetic nervous system tone to these vessels is chemically interrupted with reserpine. By treating an animal with reserpine, the normal neurotransmitters (stimuli) that are involved in regulating blood vessel tone (blood pressure) are depleted, and this is how reserpine lowers blood pressure. Thus, since stimulus intensity is decreased, blood vessels will up regulate (increase) their reponsiveness. When removed from the animal and studied in an isolated tissue bath containing a physiological saline solution, these vessels were supersensitive to selective vasoconstrictor stimuli specifically norepinephrine and Bay K 8644.

The results indicated that: 1) tissues from reserpine treated animals were selectively supersensitive to the normal neurotransmitter, norepinephrine and the selective potential-dependent Ca^{++} channel activator, Bay K 8644; 2) D600, a Ca^{++} entry blocker, eliminated the difference in sensitivity to norepinephrine; 3) no differences between treated and control were noted in the sensitivity to Ca^{++}, KCl, or histamine; 4) neither control nor reserpine treated vessels responded to beta adrenoceptor stimulation with

isoproterenol; and 5) no differences in the sensitivity to the relaxing action of acetylcholine and nitroprusside were noted in vessels from treated and control animals.

Therefore, a major portion of the enhanced sensitivity found in reserpine treated vessels is related to norepinephrine-induced activation of potential-dependent Ca^{++} channels.

INTRODUCTION

A. Vascular Smooth Muscle Morphology and Function

Vascular smooth muscle is located in the middle layer of the blood vessel wall between an adventitial layer and the endothelium. The muscle cells are primarily arranged in a circular or helical pattern. Smooth muscle lacks the striated appearance which is characteristic of both skeletal and cardiac muscle.

Vascular smooth muscle cells are shortened by the release of the neurotransmitter, norepinephrine, from postganglionic sympathetic neurons in the adventitial layer of the vascular wall. They can be contracted by norepinephrine and histamine, or relaxed by various vasodilators.

B. Sources of Ca^{++} Entry

Cytoplasmic calcium ions interact with calmodulin (Adelstein and Klee, 1981) and/or leiotonin (Ebashi, 1980) to activate smooth muscle myofilaments. Smooth muscle cells are activated either through stimulation of pharmacologic receptors located in the cell membrance or through depolarization by electrical stimuli received through low resistance junctions from adjacent smooth muscle cells. Evidence obtained to date indicates that depolarization causes the opening of membrane calcium channels, which facilitate calcium entry into the cytoplasm. Activation of

specific receptors appears to initiate a more complex set of events, which includes (1) release of surface bound calcium; (2) opening of receptor-operated calcium channels; (3) release of intracellular calcium; and (4) depolarization with a consequent opening of potential-dependent calcium channels (Flaim and Zelis, 1982). In this project, we paid special attention to two separate calcium channels: (1) those operated by membrane potential depolarization (PDCs); and (2) those operated by receptor activation (ROCs).

C. Pharmacological Studies

Chemicals that interact with a receptor and thereby initiate a cellular reaction are termed agonists. Chemicals that do not cause contraction but prevent or attenuate the interaction of an agonist, such as acetylcholine with its receptor are termed antagonists (Craig and Stitzel, 1986). On the basis of the observed selectivity of action among agonists and antagonists , it was proposed that two types of adrenoceptors exist, alpha (α) and beta (β). The α receptors respond to natural and synthetic sympathomimetic drugs by setting off an increase in activity of smooth muscle cells (an exitatory response). Thus, those smooth muscle cells in which α receptors predominate respond to an adrenergic stimulation by contracting. On the other hand, those smooth muscle cells that contain mainly β receptors, respond to adrenergic stimulation by a decrease in their contractile activity (Claborn and Wiersig, 1987).

Reserpine is a Rauwolfia alkaloid (Claborn and Wiersig, 1987). It binds to the vesicular transport system. Thus, it inhibits the transport of dopamine into the vesicle and therefore prevents dopamine's conversion to norepinephrine. It will also intefer with storage because, as norepinephrine in the vesicle gradually leaks out or is released, it cannot be transported back into the vesicle. The norepinephrine that diffuses out of the vesicles will be destroyed by mitochondrial monoamino oxidase (MAO). The result is a gradual disappearance of the transmitter from the adrenergic nerves, a phenomenon frequently referred to as transmitter depletion (Craig and Stitzel, 1986). Reserpine is the best-studied compound of this group and is also the alkaloid most frequently employed clinically (Craig and Stitzel, 1986).

1) Vasoconstrictors

Norepinephrine is the neurotransmitter produced and released in adrenergic synapses. It stimulates a specific receptor (α adrenoceptor), causing vasoconstriction. Bay K 8644 [methyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-tri-fluoro-methyl)-pyridine-5-carbaxylate] is a substance known to increase calcium influx through the potential-dependent calcium channel (Goto, Satoh, and Taira, 1985). Most tissue histamine is found in the granules of mast cells. It stimulates smooth muscle. Histamine apparently exerts its action via two types of receptors, H_1-and H_2- receptors

(Dipalma, 1976). Prostaglandin $F_{2\alpha}$ has similar mechanisms of action as norepinephrine, but works on a different membrane receptor, which will delineate specific reserpine induced changes from non-specific changes. Increased extracellular potassium causes contraction by a completely different mechanism without stimulating a specific receptor (depolarizing the cell membrane which opens channels for calcium to enter that are different than those opened by norepinephrine).

2) Vasodilators

Nitroprusside selectively blocks calcium release and calcium entry induced by norepinephrine, without binding to the norepinephrine receptor. D600 selectively blocks the calcium channel opened by potassium. Acetycholine produces vasodilation in most of the major vascular beds by releasing a vascular relaxing factor from endothelial layer. Isoproterenol is a potent stimulant of the β receptors. Phentolamine is a specific α blocker.

D) Objectives

Cells that make up different tissues and organs (effectors) have an inherent ability to regulate their own sensitivity and responsiveness (increasing or decreasing) to almost any stimuli. This is an inverse relationship, which is a function of stimulus intensity and duration. Thus, as stimulus intensity and duration increase the cell tends to decrease its own reponsiveness (down regulation). Conver-

sely, as stimulus intensity and duration decrease the cell tends to increase its responsiveness and sensitivity (up regulation). This allows the cell to adapt to chronic changes in exitatory input in order to maintain normal bodily status quo, and is therefore a basic homeostatic mechanism. The cell does this by altering membrane or cytosolic receptors that recognize a given stimulus or by varying the complex sequence of events that couple receptor signals to specific effects. Comparing differences in sensitivity (ED_{50} values) and responsiveness (maximum contractions or relaxations) between vessels from control and reserpine treated animals should indicate which mechanisms for contraction are most important in the external iliac artery (specific receptor-mediated calcium release and calcium entry or membrane depolarization-mediated calcium entry), and which of these mechanisms are affected by reserpine treatment.

METHODS

A. Tissue Preparation

New Zealand white rabbits (13-14 weeks old, 1.3-2.4 kg) that had been treated for one week with reserpine (0.3 mg/kg/day; IM) and untreated age and weight matched rabbits (control) were euthanized with sodium pentobarbital (50 mg/ml) through the lateral ear vein. A segment of the external iliac artery was removed. Each vessel segment was then placed in a cold physiological saline (PSS) solution for further dissection procedures. The composition of the PSS in mmol/l was: NaCl 154; KCl 5.4; Dextrose 11; Tris 6; $CaCl_2$ 2.0 continuously aerated with 100% O_2 (pH 7.3-7.4). Excess fat and adhering connective tissue was gently removed. Next each vessel was sectioned tranversely into shorter ring segments approximately 1.5-2.5 mm in width. Individual ring segments were then attached to two stainless steel hooks inserted through the lumen of each vessel segment. Subsequently, one hook were attached to a stainless steel rod so that the vessel segment could be placed in a 10 ml jacketed, isolated organ bath containg 10 ml of PSS. This jacketed organ bath allows appropriately warmed water to continually flow through the surrounding jacket to maintain the temperature of the PSS at normal bodily temperature, 37 degrees Celsius. The second hook is attached to a Grass FT-03 force displacement transducer that

is connected to a Gould 2600S polygraph. This transducer converts a mechanical signal (a change in the tension generated by a given vessel) into an electrical signal that is then displayed as a tracing on chart recording paper. This will allow continuous monitoring of increases (constriction of vessel segments) or decreases (relaxation or dilation of vessel segments) in vessel tone in response to selected drugs that alter vessel tone. All vessels were allowed to equilibrate in PSS for 90-120 minutes with 15 minute-rinse intervals and maintained under appropriate resting stretch before basic contraction and relaxation experiments were started. During the 90 minute equilibration interval, the vessels were contracted with a single dose of 40mM KCl 5-10 min after being hooked up for initial equilibration, then rinsed; 45 min later, they were contracted again with another single dose of 40mM KCl to determine vessel viability.

B. Experimental Protocol

To determine the optimal stretch, vessels starting at zero initial stretch were stretched in 5mm increments, equilibrated at this stretch for 15 minutes, and then stimulated with norepinephrine, and isometric tension recorded for 3-5 minutes. The agonist was rapidly rinsed from the bath. This sequence (5mm stretch, 15 min equilibration period, agonist stimulation, rinse) was repeated until the maximal developed tension was attained, which by

definition was the optimal stretch for each vessel.

After optimal resting tension was defined, dose response relationships were obtained for selected vasoconstrictors and vasodilators in vessel segments from treated and control animals. Dose response relationships were obtained by exposing individual vessel segments to cumulatively increasing concentrations of each vasoactive agent (without washing the previous concentration of agent out) until the maximal contraction (or relaxation) is attained. Before successive concentrations of each agent were added, vessel segments were allowed to reach a new steady state level of developed tension. Dose response relationships for vascular relaxants were obtained from vessel segments that were first contracted with a selected vasoconstrictor, since resting tone in these vessel segments is usually minimal.

Vasoconstrictors included: norepinephrine (Sigma), histamine (Sigma), Bay K 8644 (Miles Inst.), KCl, $CaCl_2$, Prostaglandin $F_{2\alpha}$ (Upjohn). Vasodilators included: D600 (AG Knoll), phentolamine (CIBA-GEIGY), acetylcholine (Sigma), nitroprusside (Sigma), isoproterenol (Sigma), and papaverine (Sigma). All drugs were dissolved in distill water except Bay K 8644, which was dissolved in 99% ethanol.

C. Statistical Analysis

The index to be used for describing sensitivity changes between different vessels and between vessels from treated

and control animals is the ED_{50}. This value was graphically calculated from each individual dose response relationship, and represents the concentration of drug producing 50% of the maximum response (constriction or relaxation). Mean ED_{50} values were expressed as geometric means with 95% confidence intervals. Additionally, any differences in threshold concentrations (the lowest concentration resulting in a measured change in resting tone) were also quantified, summarized, and compared in a similar manner. Changes in responsiveness were manifested as changes in the maximal constriction or relaxation in a given vessel segment.

RESULTS and DISCUSSION

A. Optimal Stretch

After letting the tissues equilibrate under an initial stretch of 5mm (0.5g tension), $3x10^{-7}$M NE was added and the maximal contraction was recorded. Vessels were rinsed for at least 30 minutes and the procedure was repeated after increasing resting tension in 0.5g increments (0.5-3.0g). The results (Figure 1) clearly show that tissues from both control and reserpine treated animals attain the maximal contraction per gram tissue at 1.5g initial tension. The following studies were conducted under these optimal stretch conditions.

B. Effects of Norepinephrine (NE)

In Figure 2, there is a nice shift to the left of dose response curve (DRC) and a concomitant decrease in ED_{50} (the effective dose that causes 50% total contraction) for NE in vessels from treated animals as compared to control vessels. This shift in the DRC is reflected in an approximate 3-fold increase in control ED_{50} values as compared to the treated ED_{50} values. The maximal developed tensions of the control and treated animals were: control, 5.03 ± 0.3g; treated, 4.92 ± 0.2g.

Because the pathways of Ca^{++} entry in NE-induced responses are primarily via (1) potential dependent channels or (2) receptor-operated channels, the enhanced responsiv-

Figure 1

Summary of results showing the effects of increasing initial tension on responses to $3x10^{-7}$M NE. Vertical bars indicate ± S.E.M. of the force developed, n=8.

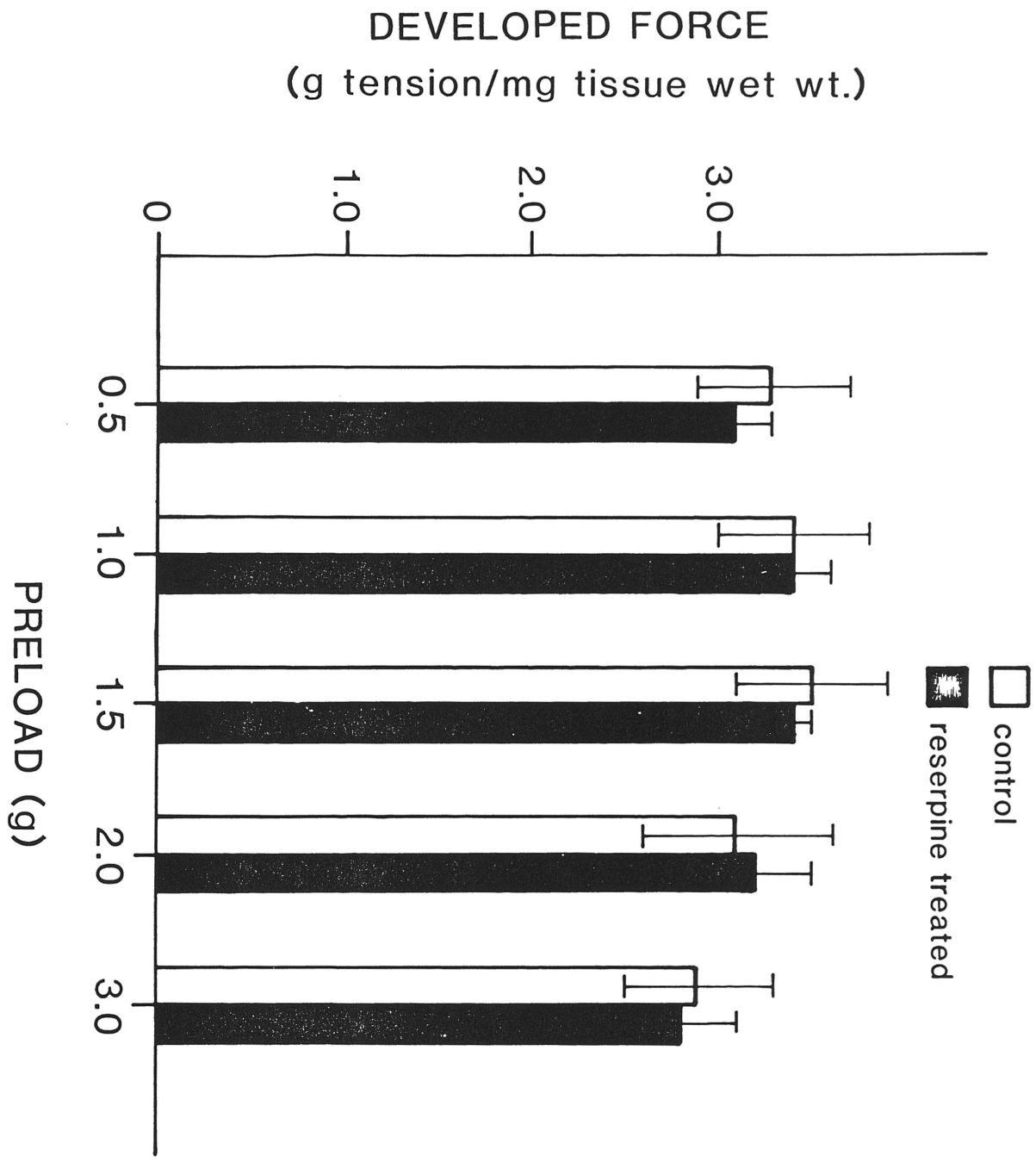
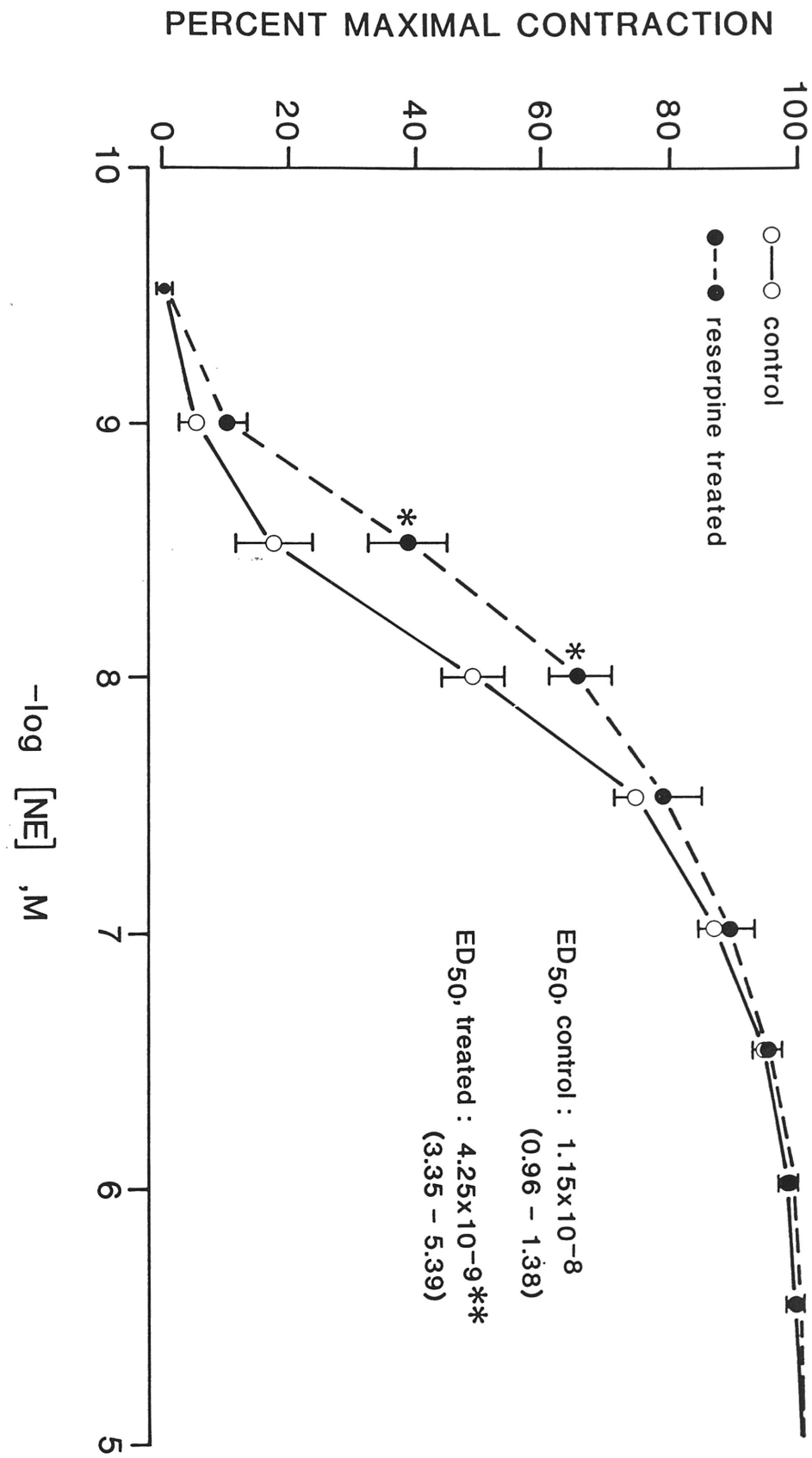

DEVELOPED FORCE
(g tension/mg tissue wet wt.)
0
1.0
2.0
3.0
PRELOAD (g)
0.5
1.0
1.5
2.0
3.0
control
reserpine treated

Figure 2

Dose response curves for NE in vessels from control and reserpine treated animals. Mean values ± S.E.M., n = 13-16 for each drug concentration.
* $P < 0.05$
** $P < 0.005$


PERCENT MAXIMAL CONTRACTION
100
80
60
40
20
0
control
reserpine treated
*
*
ED50, control : 1.15x10-8
(0.96 - 1.38)
ED50, treated : 4.25x10-9**
(3.35 - 5.39)
10
9
8
7
6
5
-log [NE] ,M

eness of reserpine treated vessels could be related to an alteration in Ca^{++} entry.

C. Effects of Bay K 8644

Bay K 8644 is a substance known to selectively increase Ca^{++} influx through potential-dependent Ca^{++} channels. With Bay K 8644, there was also a nice shift to the left in the DRC and decrease in the ED_{50} of the treated vessels compared to control vessel (Figure 3). The ratio of ED_{50} of control to ED_{50} of treated is 2.80, which was similar to the increase seen with NE. The maximal developed tension of control and reserpine treated vessels were not significantly different (control, 4.1±0.2g; treated, 3.7±0.4g). Since the sensitivity increase is similar, the enhanced sensitivity to NE is possibly related to a reserpine related effect on potential-dependent channels.

D. Effects of NE in the presence of D600

D600, a selective inhibitor of potential-dependent Ca^{++} channels, eliminates the supersensitivity of the vessels from treated animals to NE (Figure 4). It means that in the presence of D600 both control and treated animals require the same amount of NE to elicit 50% of the maximal developed tension ($ED_{50}C/ED_{50}T=1.06$). The maximal contractions were similar in both tissues (control, 4.8±0.2g; treated, 5.0±0.4g).

Figure 3

The effect of Bay K 8644 on external iliac arteries from control and reserpine treated animals. Data are presented as means ± S.E.M. (n=13).
* $P < 0.05$
** $P < 0.002$

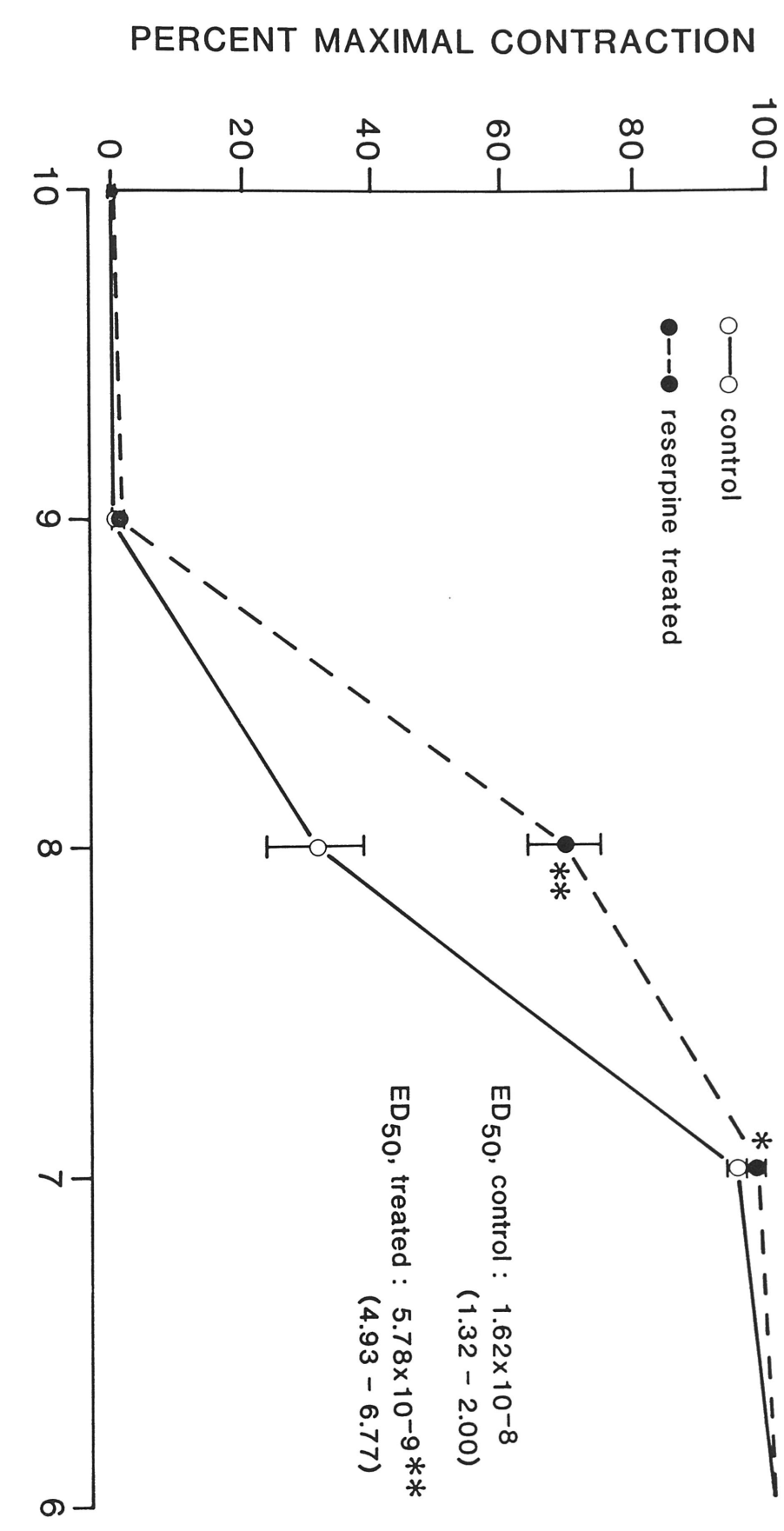
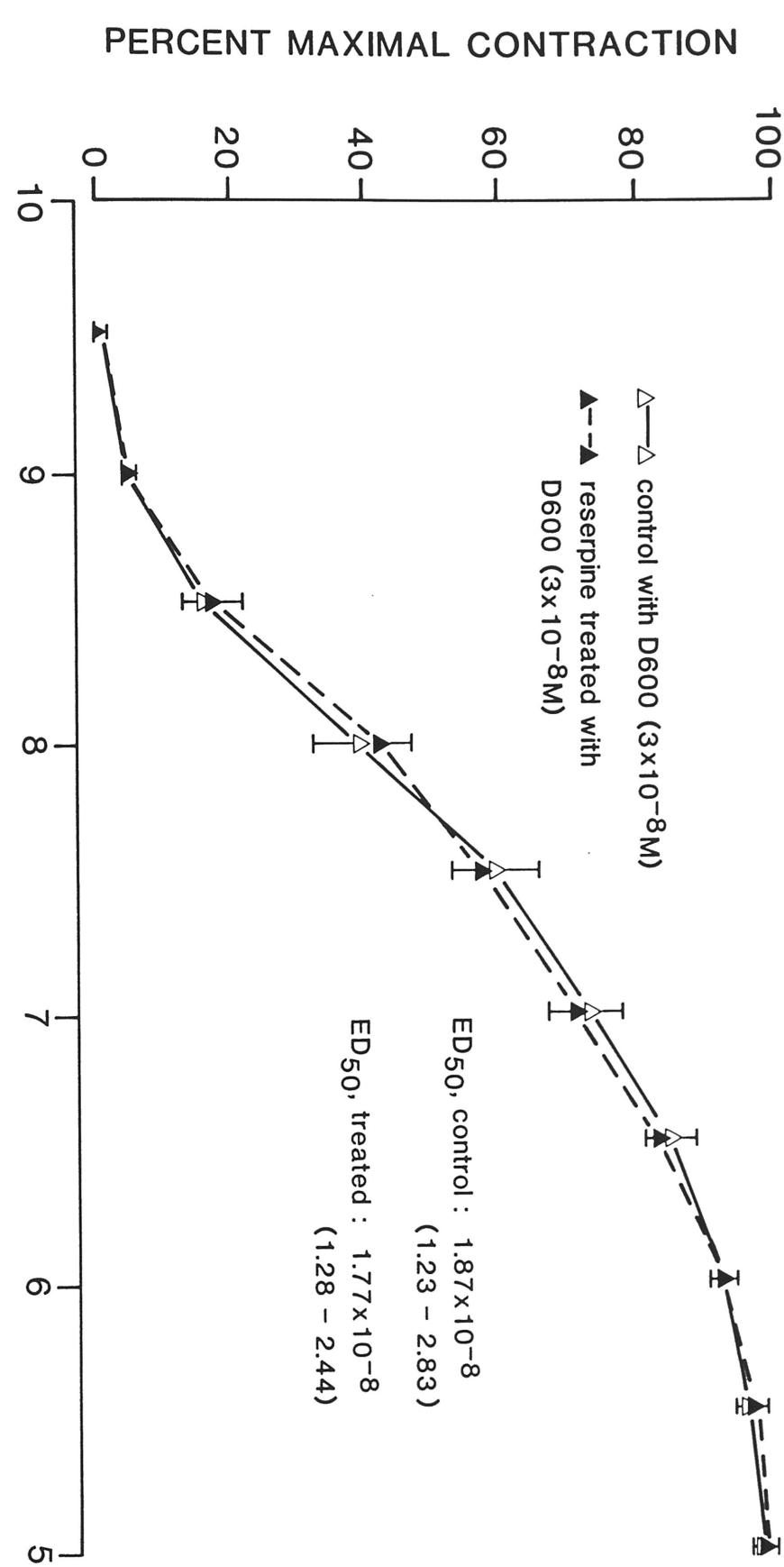

PERCENT MAXIMAL CONTRACTION
100
80
60
40
20
0
control
reserpine treated
*
**
ED50, control : 1.62x10-8
(1.32 - 2.00)
ED50, treated : 5.78x10-9**
(4.93 - 6.77)
10
9
8
7
6
-log [Bay K 8644] ,M

Figure 4

Dose response curves for NE in vessels from control and reserpine treated animals in the presence of D600 ($3x10^{-8}M$), an inhibitor of potential-dependent Ca^{++} channels. Data are plotted as means ± S.E.M. (n=7).


PERCENT MAXIMAL CONTRACTION
100
80
60
40
20
0
10
9
8
7
6
5
-log [NE] ,M
△—△ control with D600 (3x10⁻⁸M)
▲--▲ reserpine treated with D600 (3x10⁻⁸M)
ED50, control : 1.87x10⁻⁸
(1.23 - 2.83)
ED50, treated : 1.77x10⁻⁸
(1.28 - 2.44)

E. Effects of Other Vasoconstrictors: histamine, KCl, and Ca^{++}

Histamine, KCl, and Ca^{++} which are drugs that have different mechanisms of action than NE respond similarly in vessels from control and reserpine treated animals. There were no differences in sensitivity to these agonists between the control and treated animals. The ED_{50} values in control vessels were 8.72×10^{-7}M, 25.8mM, 1.30mM and in treated vessels were 6.44×10^{-7}M, 19.2mM, 1.15mM, respectively, for histamine, KCl and Ca^{++}.

F. Effects of vasodilators: nitroprusside, acetylcholine, and isoproterenol

There is also no difference in the sensitivity to these vascular relaxants in control and treated animals. ED_{50} values of control and treated vessels were, 3.45×10^{-6}M and 1.76×10^{-6}M, respectively, for nitroprusside-induced relaxation of 10^{-5}M histamine-induced contractions. ED_{50} values of control and treated vessels for acetylcholine-induced relaxation of 4.1×10^{-6}M prostaglandin $F_{2\alpha}$ contractions were 1.46×10^{-7}M, and 4.24×10^{-7}M, respectively. There is no relaxation of prostaglandin $F_{2\alpha}$ precontracted vessels with isoproterenol indicating an absence of beta adrenoceptors in the external iliac artery.

CONCLUSIONS

Reserpine-induced supersensitivity appears to be related to a specific effect on NE-induced activation of potential-dependent Ca^{++} channels as opposed to receptor-operated Ca^{++} channels since the tissues were also supersensitive to Bay K 8644, a selective potential-dependent Ca^{++} channel activator. Additionally, the enhanced sensitivity to NE was eliminated by the selective Ca^{++} channel antagonist, D600. This enhanced sensitivity was apparently selective for the normal neurotransmitter, NE, since there were no differences in the sensitivity to histamine, KCl, or Ca^{++}.

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