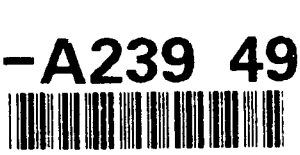

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REPORT NO T11-91

COLD-INDUCED CHANGES IN ARTERIAL SENSITIVITY

U S ARMY RESEARCH INSTITUTE
OF
ENVIRONMENTAL MEDICINE
Natick, Massachusetts

MAY 1991

UNITED STATES ARMY
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Technical Report T11-91

COLD-INDUCED CHANGES IN ARTERIAL SENSITIVITY

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We studied the effects of cold on rabbits and pigs: 1) to determine how cold affects the smooth muscle sensitivity to receptor-mediated (NEPI) or nonreceptor-mediated (KCL) (pigs only) agonist induction of in vitro vascular contraction; 2) to determine whether cold-induced vascular sensitivity to catecholamines exists in an intact hypothermic pig model; 3) to determine the effect of cortisol on the arterial smooth muscle contraction. The in vitro exposure of femoral arteries from rabbits and pigs to cold resulted in a progressive loss of sensitivity to agonist. Femoral arteries isolated from hypothermic pigs (core temp = 25° C for 2 hours) were no more sensitive to NEPI in vitro than arteries from normothermic animals. However, the in situ hind limb arterial bed of the hypothermic pig was ten times more sensitive to arterial injection of NEPI than the arterial bed of the normothermic pig. The sensitivity of porcine vascular smooth muscle to NEPI does not appear to be affected by cortisol. These data suggest that cold evokes an extravascular control (mechanisms not intrinsic to vascular tissue) over NEPI sensitivity. Moreover, these data suggest that the mechanism for control of cold-induced sensitivity to sympathetic neurotransmitter in the pig is different from the mechanism which operates in the rabbit.

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EXECUTIVE SUMMARY

Vascular tissue removed from hypothermic rabbits is more sensitive to norepinephrine (NEPI) than vascular tissue from normothermic rabbits. Since it is known that cortisol attenuates the re-uptake of NEPI, the cold-induced release of cortisol could be a possible mechanism for the enhanced contractile response following hypothermia. We studied the effects of cold on rabbits and pigs: 1) to determine how cold affects the smooth muscle sensitivity to receptor-mediated (NEPI) or nonreceptor-mediated (KCL) agonist (pigs only) induction of in vitro vascular contraction; 2) to determine whether cold-induced vascular sensitivity to catecholamines exists in an intact hypothermic pig model; 3) to determine the effect of cortisol on the arterial smooth muscle contraction. The in vitro exposure of femoral arteries from rabbits and pigs to cold resulted in a progressive loss of sensitivity to NEPI and to KCL (pigs). Femoral arteries isolated from hypothermic pigs (core temp = 25° C for 2 hours) were no more sensitive to NEPI in vitro than arteries from normothermic animals. However, the in situ hind limb arterial bed of the hypothermic pig was ten times more sensitive to arterial injection of NEPI than the arterial bed of the normothermic pig. The sensitivity of porcine vascular smooth muscle to NEPI does not appear to be affected by cortisol. These data suggest that cold evokes an extravascular control (mechanisms not intrinsic to the vascular tissue itself) over NEPI sensitivity. Moreover, these data suggest that the mechanism for control of cold-induced sensitivity to sympathetic neurotransmitter in the pig is different from the mechanism which operates in the rabbit.

INTRODUCTION

Acute episodes of cold exposure result in profound peripheral vasoconstriction (1,2,3,4,5). The mechanism by which such sustained periods of vasoconstriction can be maintained (6,7,8), and vasoconstrictor regulation at both local and central sites (9,10,11) have been considered. Several reports considered the increased sensitivity of vascular smooth muscle to adrenegic agonist to be a likely explanation for some of this vasoconstriction of superficial blood vessels (8,12,13). This was considered by some to be a relatively short-lived increase in norepinephrine sensitivity, which was lost when tissues were rewarmed to 37° C (13,14). Bandick, et al. observed increases in the in vitro sensitivity of vascular rings from hypothermic rabbits to norepinephrine (15). These changes persisted for 8-12 hours, even after the vascular tissues were rewarmed and washed in a 37° C bath. The fact that this change required in vivo exposure to cold suggests an extravascular control (mechanisms not intrinsic to the vascular tissue itself) over long-lasting changes in the catecholamine sensitivity of arterial smooth muscle. Moreover, the fact that cold-induced increase in in vitro sensitivity to norepinephrine persist for hours, even in the face of several washes, suggests that relatively stable changes were induced in the pharmaco-mechanical coupling of the contractile apparatus. While it is not known with certainty, these changes may have been induced by extravascular controls.

A cold-induced vasoconstriction, a calcium requiring event, is in itself somewhat of an an anomalous phenomenon. Abundant evidence indicates that extracellular calcium is required to support contractile events in vascular smooth muscle (16,17). With agonist-induced membrane depolarization, extracellular calcium enters the cell through both voltage-dependent and voltage-independent channels (18,19,20). The calcium which enters in this manner, may also mediate intracellular calcium release (16,21) required for significant activation of the contractile filaments and resulting in contraction. Following contraction, calcium is released from contractile filaments and may find its way to a "sink", which is different from the original calcium source (22). This calcium eventually exits the cell and is once again available to participate in the calcium cycle. Alternatively, Casteels and associates presented evidence that some calcium which is stored within the vascular smooth muscle is available for contraction (23). This evidence comes from prolonged stimulation of vascular muscle which is bathed in a calcium-free medium. However, they utilized aortic tissue, the stimulus was a single pharmacologic dose of NEPI, and the response appeared to wane with time. Whereas in cold-incubated, intact vasculature, the adrenergic stimulus is presumed to be rather constant and prolonged. This tends to indicate that the majority of the

calcium required to support cold-induced vasoconstriction is derived from an extracellular source. However, since plasmalemmal permeability to many substances, including calcium, is considerably reduced or abolished with low temperature (24), it is unclear how profound vasoconstriction (a calcium current-requiring event) can occur or be maintained in the face of such reduced calcium currents.

Cold exposure is a stress which mediates the release of adrenal hormones common to the stress reaction (25). Little is known about how these hormones may affect receptor-mediated or non-receptor-mediated initiation of contraction in arterial smooth muscle, other than in the case of catecholamine responses. There is some evidence to suggest that the presence of cortisol prolongs the extracellular concentration of norepinephrine by reducing its re-uptake (26). In the preponderance of animal models , the principal stress steroid is corticosterone; however, in man and in the pig, the principal stress steroid is cortisol.

The role of cortisol in cold-induced changes in vascular sensitivity is unknown. Therefore, the rationale for conducting the experiments in the present study was threefold: first, to determine whether cold per se affects the sensitivity to receptor-mediated, as well as non-receptor-mediated agonist induction of isolated (in vitro) vascular contraction; second, to determine whether a cold-induced extravascular control exists in an animal cardiovascular system which may be representative of the human system; third, to determine whether or not cortisol (at stress levels) had any effect on receptor-mediated or non-receptor-mediated contraction of arterial smooth muscle.

MATERIALS AND METHODS

ANIMAL MODEL

The rabbit has been a standard animal model for hypothermic research. Thirty New Zealand White rabbits (3-6 kg) were used as one source of tissue for these studies. Since the standard farm pig is an appropriate model of human physiology in general, and human vascular physiology in particular, arterial tissue from male Yorkshire pigs (fifteen) weighing 20-30 kg were also evaluated.

VASCULAR TISSUE

When rabbit tissue was required, bilateral femoral and carotid arteries were removed from rabbits after euthanasia by exposure to 100% CO_2. The vascular tissue was stored in Tyrode's buffer (30-60 minutes) at room temperature (20-22° C) until placement in tissue baths. Pigs were anesthetized with halothane and room air, exsanguinated and dissected to remove various arterial segments, including femoral, carotid and coronary arteries. These were stored in Tyrode's buffer (30-60 minutes) at room temperature (20-22° C) until placement in tissue baths (4,24 or 37° C). All arterial segments were trimmed to remove adventitia and cut into rings (1-3 mm wide) as needed.

When hypothermic-derived pig arterial tissues were required, pigs were anesthetized with halothane, intubated with an endotracheal tube to aid respiration, and switched to pentobarbital anesthesia (20 mg/kg IV). These pigs were cooled at 2° C per hour to 25° C with the aid of cooling blankets, which circulated a mixture of water and propylene glycol. After core temperature had remained at 25° C for 2 hours, sections of femoral artery were removed for in vitro contraction experiments.

TISSUE SENSITIVITY

In Vitro: Rings of arterial tissue were suspended in glass organ baths, between an anchor hook and a force transducer (FT 0.03; Grass Instrument Co., Cambridge, MA), using 4-0 surgical steel wire and 5-0 silk suture. These rings were bathed in physiological saline solution (PSS) containing NaCl (119 mM/L), KCL (4.7 mM/L), KH_2PO_4 (1.18 mM/L), $MgSO_4$ (1.17 mM/L), $NaHCO_3$ (14.9 mM/L), dextrose (5.5 mM/L), sucrose (50 mM/L), $CaCl_2$ (1.6 mM/L) and calcium disodium EDTA (0.026 mM/L). Unless otherwise stated, the bath temperature was 37°C. Tissues were pre-loaded (2g for rabbit; 3g for pig) in order to assess function at or near the peak of the load. These values for optimal pre-load were determined from length-tension curves (Figures 1,2). Tissues were equilibrated for 1-2 hours and pre-tested for responsiveness to a standard dose of norepinephrine (NEPI) or KCl (pigs only). Cumulative dose-response curves were constructed using either NEPI (a receptor-mediated agonist) or KCl (a non-receptor-mediated agonist).

Data were collected using a physiological recording system (Grass Instrument Co., Cambridge, MA) or a data logging system connected by interface to an IBM PC/XT computer (Buxco Electronics., Sharon, CT). These data were recorded as grams of tension or as

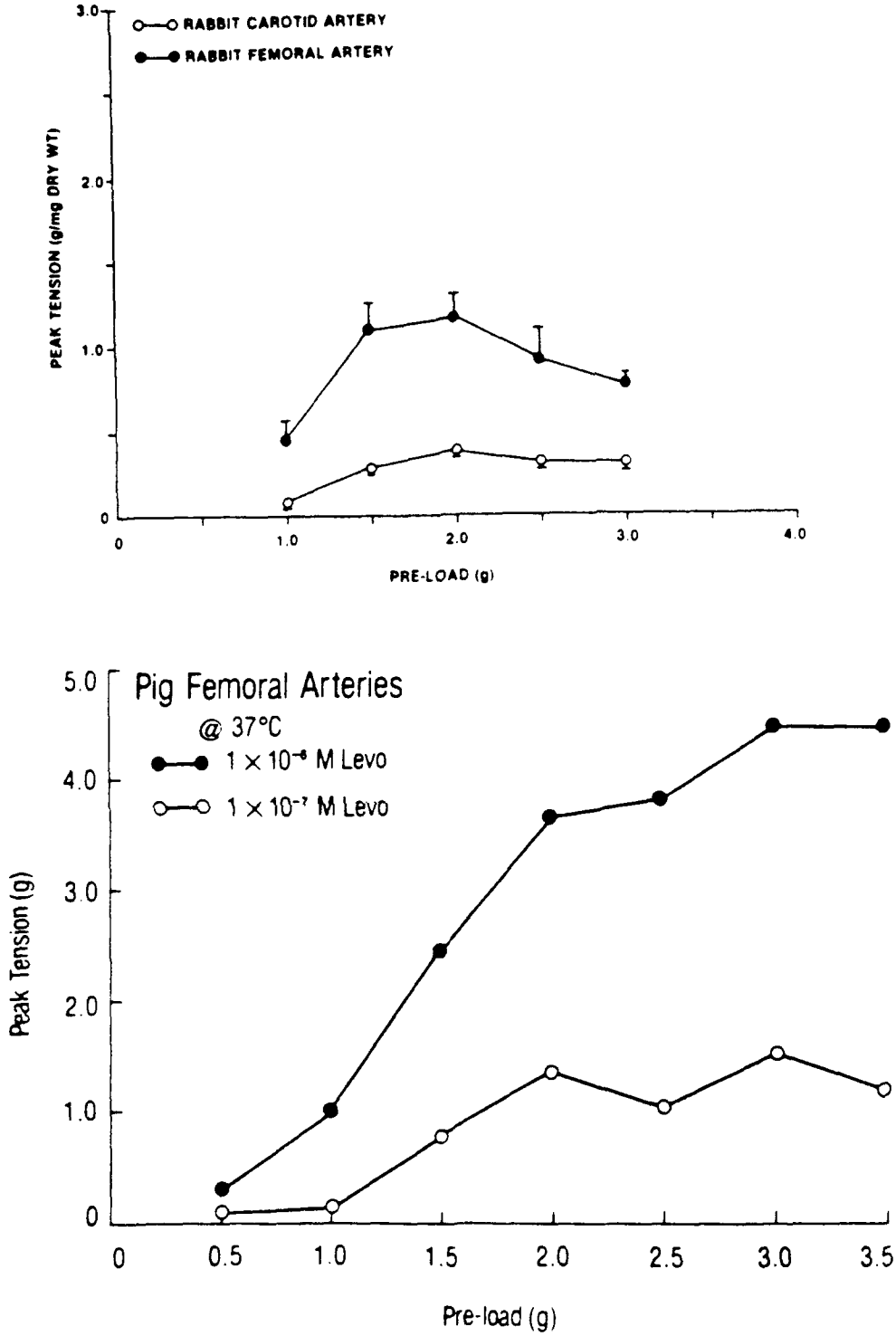

Figure 1. Length-tension curves for vascular rings from both rabbits and pigs. Length was adjusted by varying pre-load. Contractile agonist was norepinephrine (Levo). Upper panel: Length-tension curve for rabbit arteries. The peak of the curve for both types of vessel was 2.0 g of pre-load (studies conducted at 37° C). Lower panel: Load-tension curve for pig arteries. The peak of the curves was at or near 3.0 g pre-load. Values are means ± SEM.

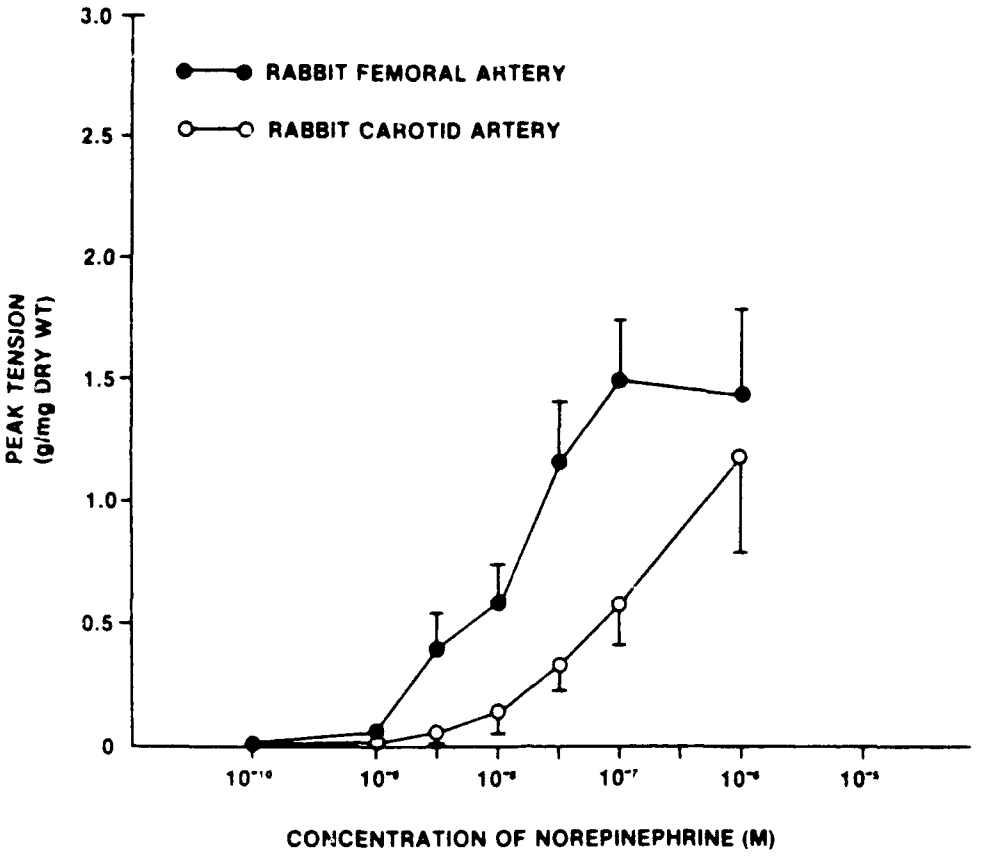

Figure 2. Dose response curve for norepinephrine in rabbit arteries. Studies were conducted at 37° C. Threshold was in nanomolar range. Values are means ± SEM.

grams of tension per milligram dry weight of tissue. Sensitivity of a tissue to an agonist was determined as a function of either the threshold dose eliciting a contractile response, the EC_{50} (the concentration which effects 50% of a maximal contraction), or both.

In Vivo: Pigs were anesthetized with halothane and intubated with an endotracheal catheter, in case assistance with ventilation was necessary. Halothane anesthesia was discontinued, and light barbiturate anesthesia was maintained, as necessary, with sodium pentobarbital (approximately 10 mg/kg). A rectal temperature probe was inserted to a depth of 5-6 inches to obtain a measure of core temperature. The external femoral arteries of both hind limbs were cannulated with 23 gauge needles connected to arterial pressure transducers by polyethylene tubing. Arterial pressure was measured from both cannulae, one site was used for NEPI injection and the other site (a control for non-bed-specific hemodynamic changes) was used for injection of an equal volume of normal saline. An

immediate rise in ipsilateral diastolic pressure (as measured with a needle cannula connected to a Statham pressure transducer) in response to close-arterial agonist injection (with no concommittant rise in either contralateral limb blood pressure or in heart rate) was taken as the measure of vascular response (contraction) to agonist, and the lowest dose to cause a response was termed the threshold dose. Threshold levels for NEPI were easily determined, but dose-response curves were affected by residual effects of previous doses.

Each animal served as its own control since threshold for NEPI was determined at a rectal temperature of 37° C, and following cooling to a rectal temperature of 25° C. Cooling was effected with two water-jacketed blankets. The temperature was controlled by a circulating water bath containing a mixture of water and propylene glycol. All animals cooled in this manner were cooled at a rate of approximately 2° C per hour, and were maintained at a rectal temperature of 25° C for 2 hours before a determination of hypothermic threshold response to NEPI was made.

EQUILIBRATION GAS MIXTURE

All vascular tissues were equilibrated using 20% O_2, 5% CO_2, 75% N_2 (which generates a PO_2 near 140 mm Hg) and was more physiologically appropriate than 95% O_2 (which has a partial pressure of oxygen in excess of 700 mm Hg) as commonly used. The normal vascular lumen PO_2 approaches 100 mm Hg.

DATA ANALYSES

Using the software support provided with the Buxco data logging system, in vitro dose-response curves were constructed, grouped and averaged. Curves were compared and tested for threshold, EC_{50} or maximum response by unpaired Student's "t" test or by Dunnett's test. In vivo threshold values were grouped, averaged and tested by paired "t" test. Statistical significance was evaluated at $P < 0.05$.

RESULTS

EFFECTS OF COLD ON AGONIST-INDUCED CONTRACTIONS IN VITRO

Cold incubation severely diminished the ability of arterial vascular tissue to respond to

contractile agonist. Pig arterial rings would not respond to either norepinephrine (a receptor specific agonist) or potassium chloride (a non-receptor-specific agonist) at 4° C (Figures 3,4,5). As a result, the ability to develop tension was lost as the tissue bath (thus the tissue itself) became colder and colder. Furthermore, when cold-incubated arterial tissues were

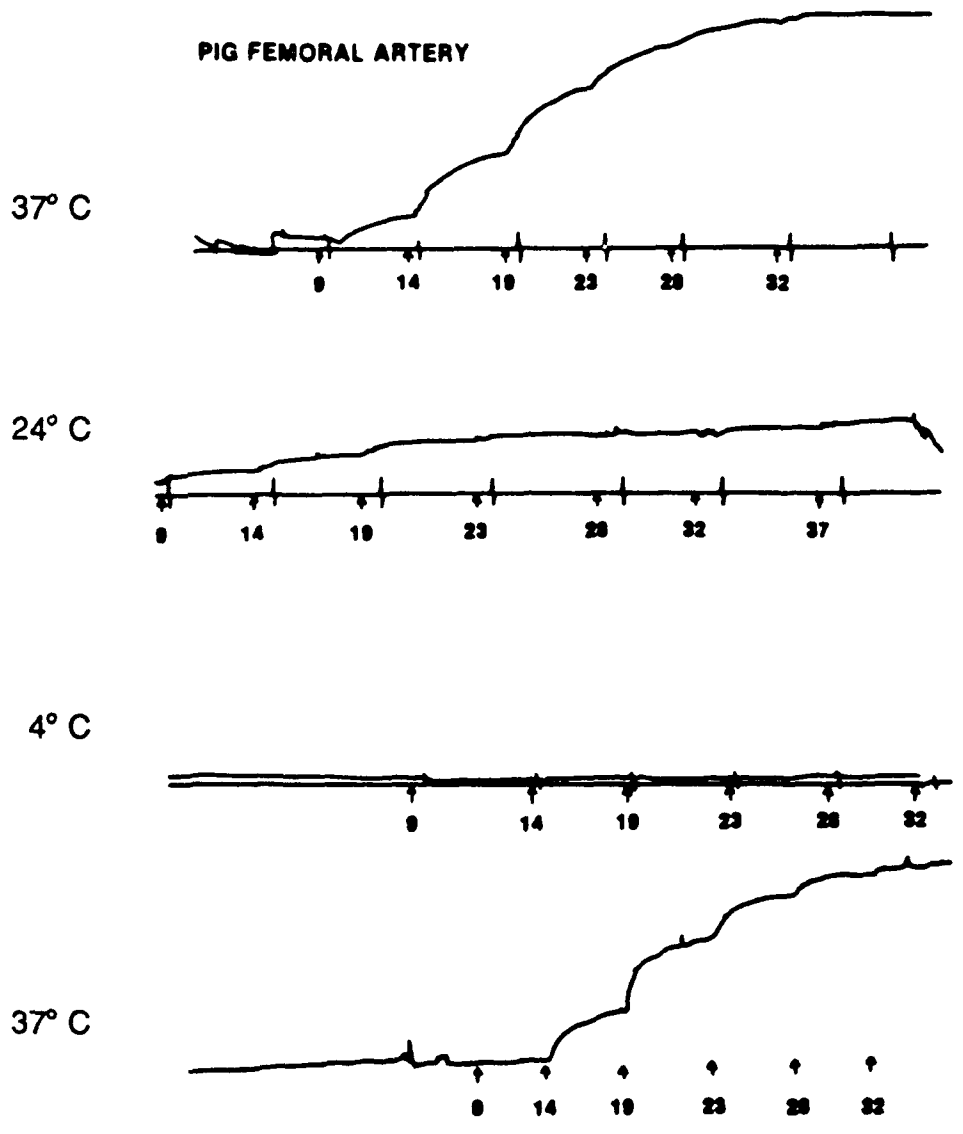

Figure 3. Typical response of pig femoral arteries to contractile agonist (KCL), and the change in response as a result of changing bath temperature. Tissues were equilibrated at least 15 minutes at each new bath temperature. Values and arrows indicate the amount of agonist (millimoles) and the point of administration.

rewarmed, the sensitivity to agonist returned towards control levels (Fig. 3,6).

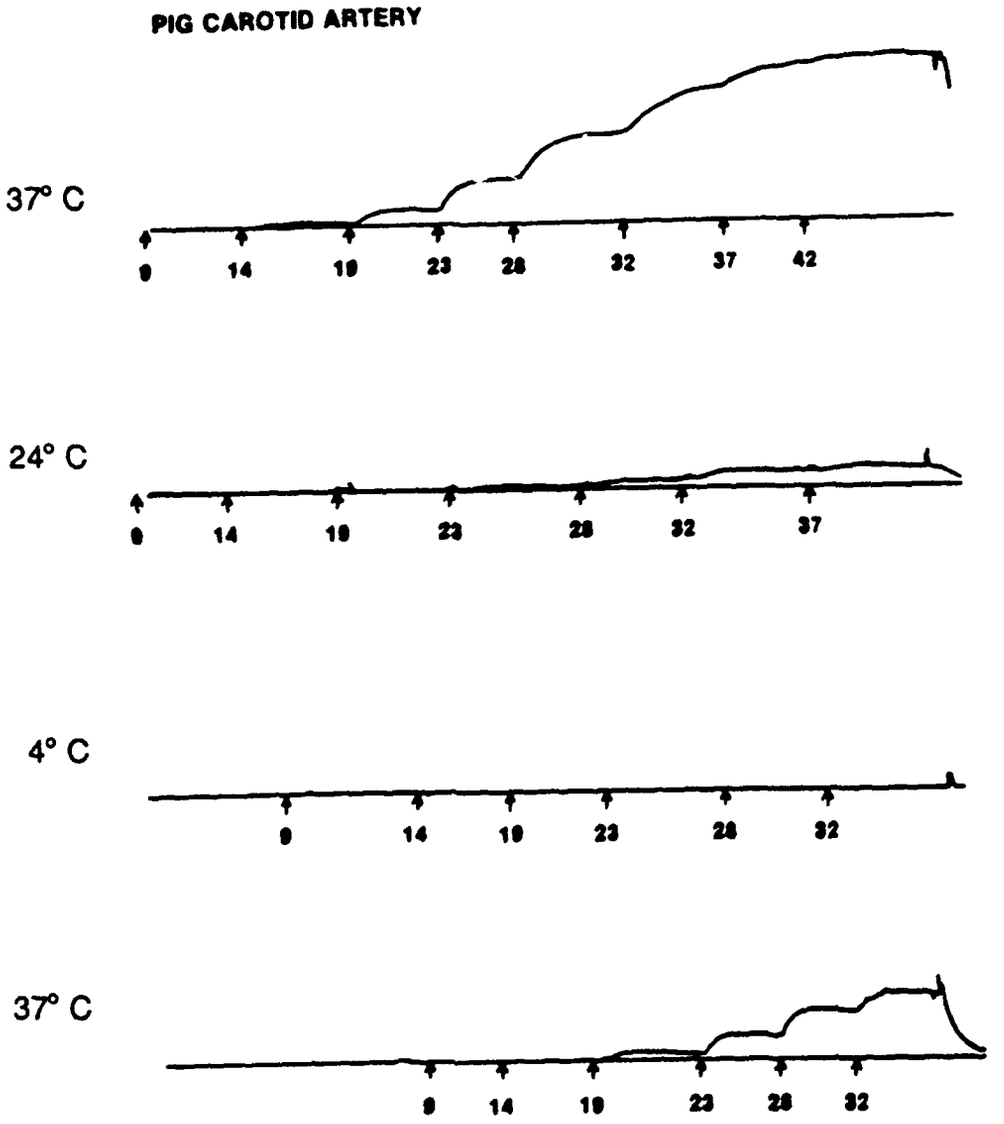

Figure 4. Typical response of pig carotid arteries to contractile agonist (KCL), and the change in response as a result of changing bath temperature. Tissues were equilibrated at least 15 minutes at each new bath temperature. Values and arrows indicate the amount of agonist (millimoles) and the point of administration.

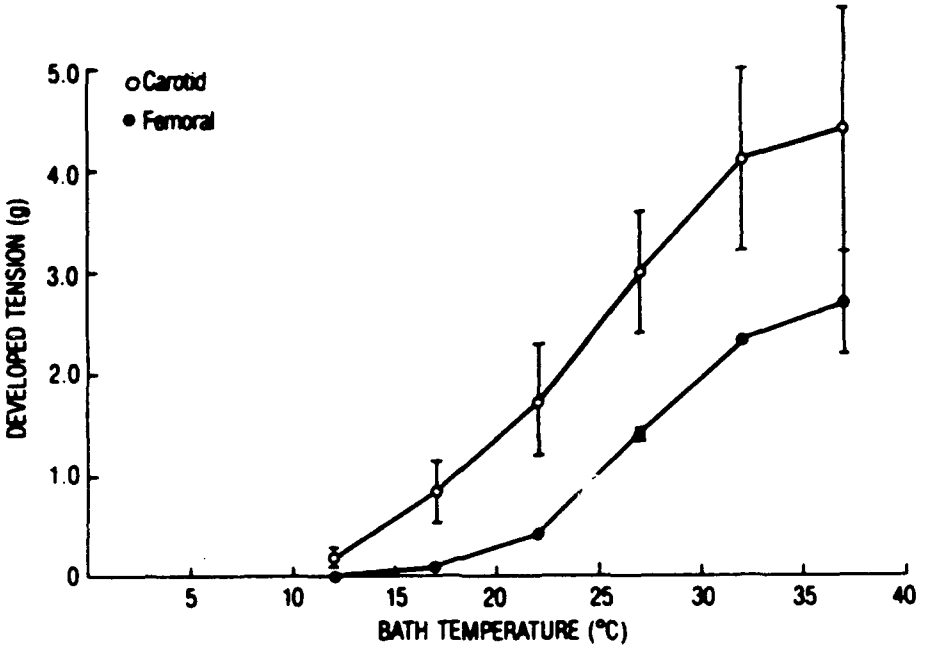

Figure 5. Summary "dose-response" curve for agonist-induced contraction of pig arteries at various incubation temperatures. Note that the "ED_{50}" was approximately 25° C for both types of arteries. Values are means ± SEM, for 3-10 arteries of each type.

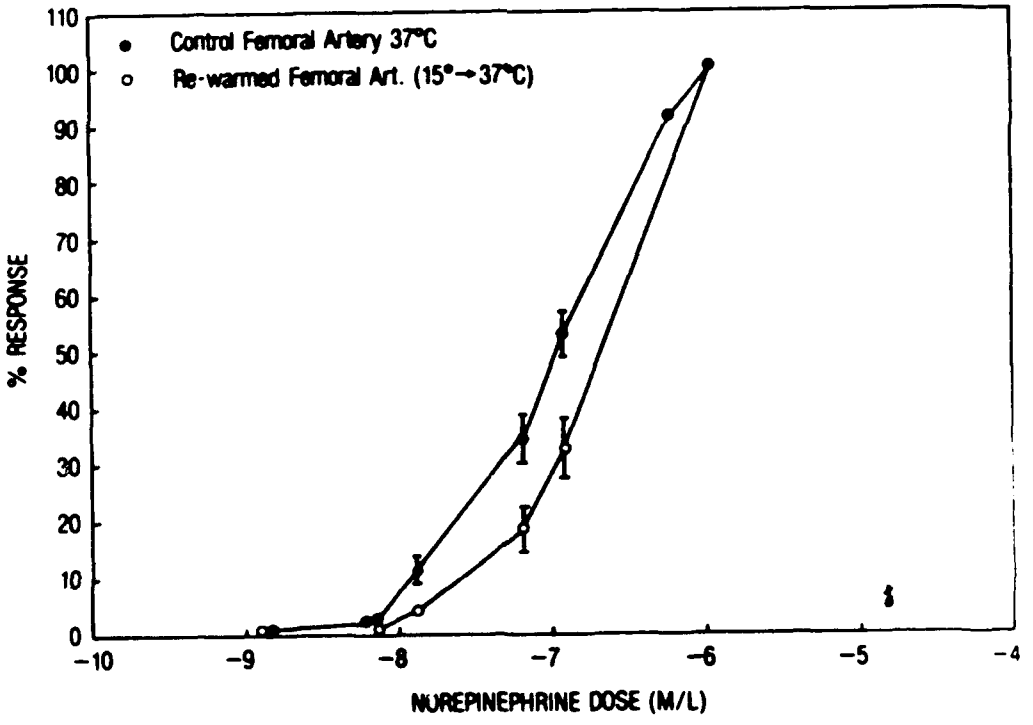

Figure 6. Dose-response curves for pig femoral artery under control conditions (37° C incubation), and after cooling (15° C for 30 minutes) followed by rapid rewarm (37° C for 15 minutes). Values are means ± SEM, for 3-10 arteries.

COLD-INDUCED RELAXATION OF VASCULAR RINGS IN VITRO

Using multiple tissue samples from the same animal, passive tension applied to both porcine femoral and porcine carotid vascular rings decreased considerably, as a function of progressively decreasing incubation temperature (Fig. 7). This tension returned partially toward control levels upon rewarming to 37° C; however, the recovery was far from complete.

SENSITIVITY OF HYPOTHERMIC PIG-DERIVED VASCULAR RINGS IN VITRO

While normothermic-derived arterial tissue responded in a predictable manner to receptor-specific agonist at 37° C (Fig. 8), it was somewhat surprising that hypothermic-derived tissue also responded in the same fashion (Fig 8). Both the amount of tension developed and the ED_{50} for the agonist were essentially the same for both tissues.

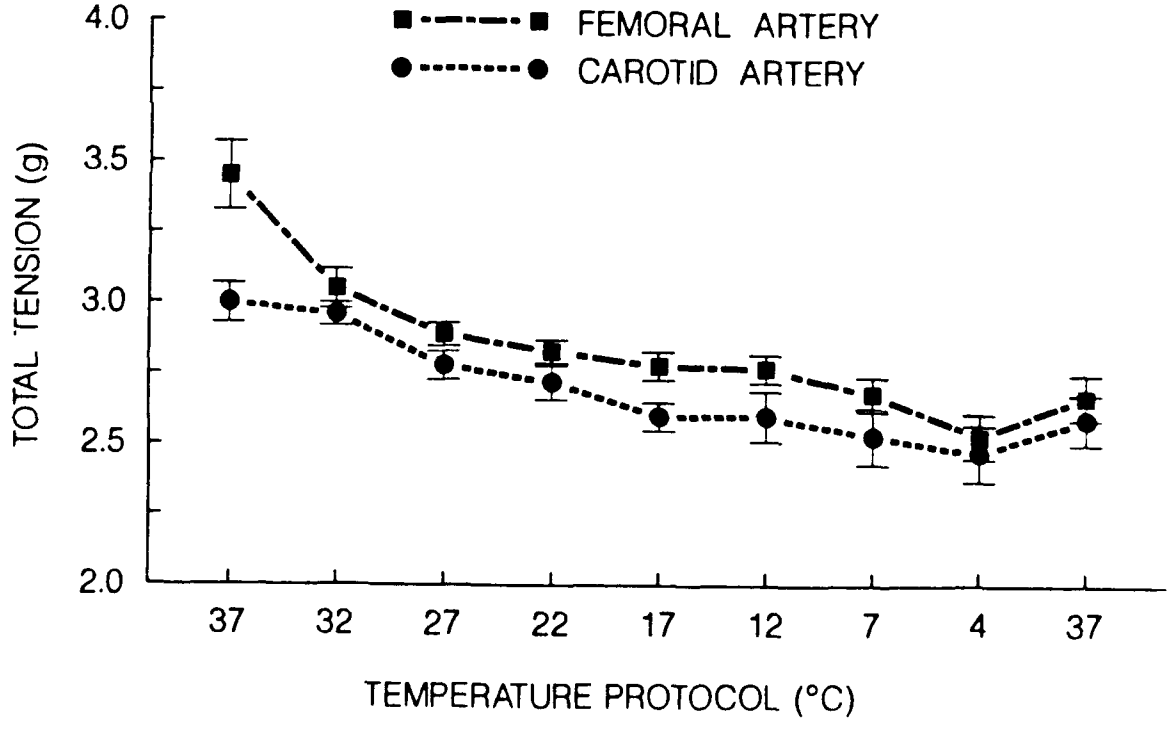

Figure 7. The effect of temperature on developed tension in pig femoral and carotid arteries.

SENSITIVITY OF NORMOTHERMIC AND HYPOTHERMIC VASCULAR TISSUE IN VIVO

The in vivo sensitivity of arterial vascular tissue was assessed by close-arterial injection of norepinephrine into a branch of the external femoral artery, which travels along the medial aspect of the knee. In these experiments, the normothermic threshold response to agonist was determined (Fig. 9), and then the animal was cooled to 25° C. After two hours at a core temperature of 25° C, the in vivo threshold response was again determined (Fig. 9). During hypothermia, the in vivo threshold sensitivity to norepinephrine was always increased substantially (Fig. 9). This was true even though "resting" systolic pressure and pulse pressure were marginally decreased from normothermic levels.

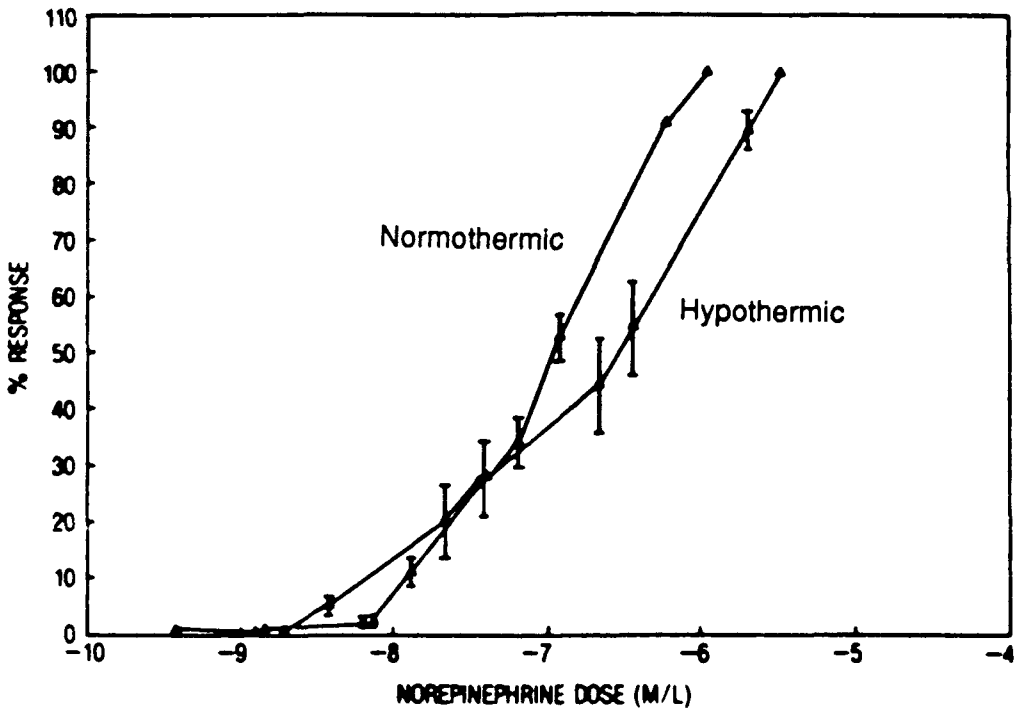

Figure 8. Dose-response curves for pig femoral artery. Arteries were removed from normothermic (37° C) animals and from hypothermic (25° C for 2 hours) animals. Values are means ± SEM for 3-10 arteries.

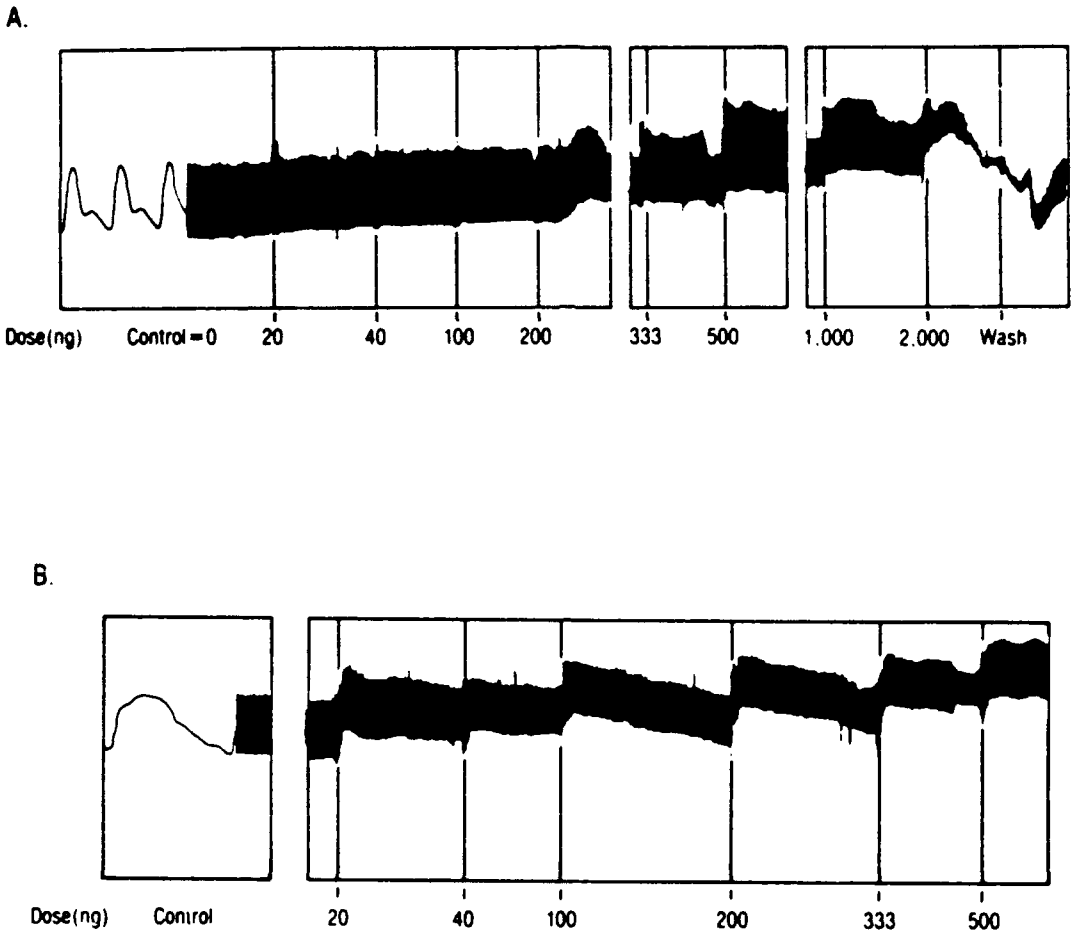

Figure 9. Typical responses of the pig hind-limb vascular bed to close-arterial injections of norepinephrine. Panel A. In vivo normothermic (temp > 36.5° C) arterial threshold response to norepinephrine. A well-defined, immediate rise in hind-limb diastolic pressure was not observed until a dose of 500 ng agonist was applied. Panel B. The same animal as in panel A. In vivo, hypothermic (temp = 25° C) arterial threshold response to norepinephrine. A well-defined, immediate rise in hind-limb diastolic pressure was observed when the lowest dose (= 20 ng) of agonist was applied. In this animal, hypothermia caused > 25X increase in arterial sensitivity to norepinephrine.

EFFECTS OF CORTISOL ON AGONIST-INDUCED CONTRACTION IN VITRO

Cortisol enhanced the response to receptor-specific contractile agonist in rabbit arterial tissue (Fig. 10). While 25 µg/dl appeared effective only with carotid arterial tissue, 40 µg/dl

was effective in both arterial tissues. However, the same dose (i.e., 40 µg/dl) was without effect in pig arterial tissue, when stimulated to contract with either receptor-specific or non-receptor-specific agonist (Fig. 11).

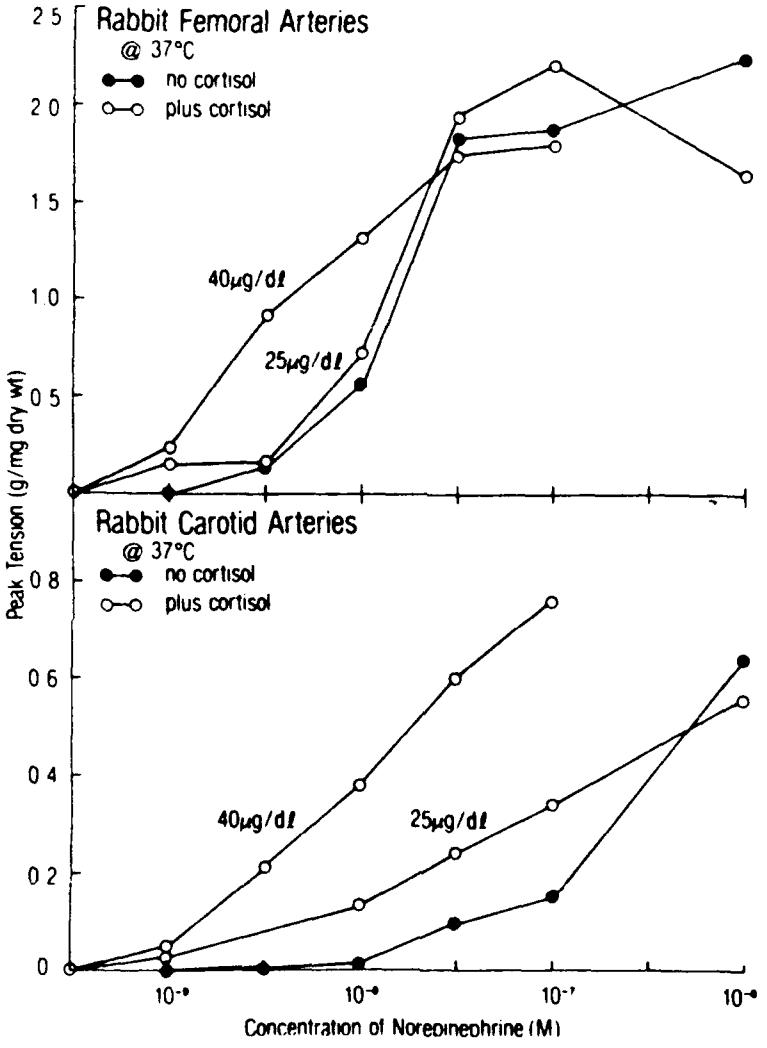

Figure 10. Dose-response curves for rabbit arteries in the presence and absence of stress steroid. Both types of arteries appear to show increased sensitivity to norepinephrine in the presence of cortisol (40 ug/dl), with carotid artery showing the greatest effect. Values are means ± SEM for 3-6 arteries.

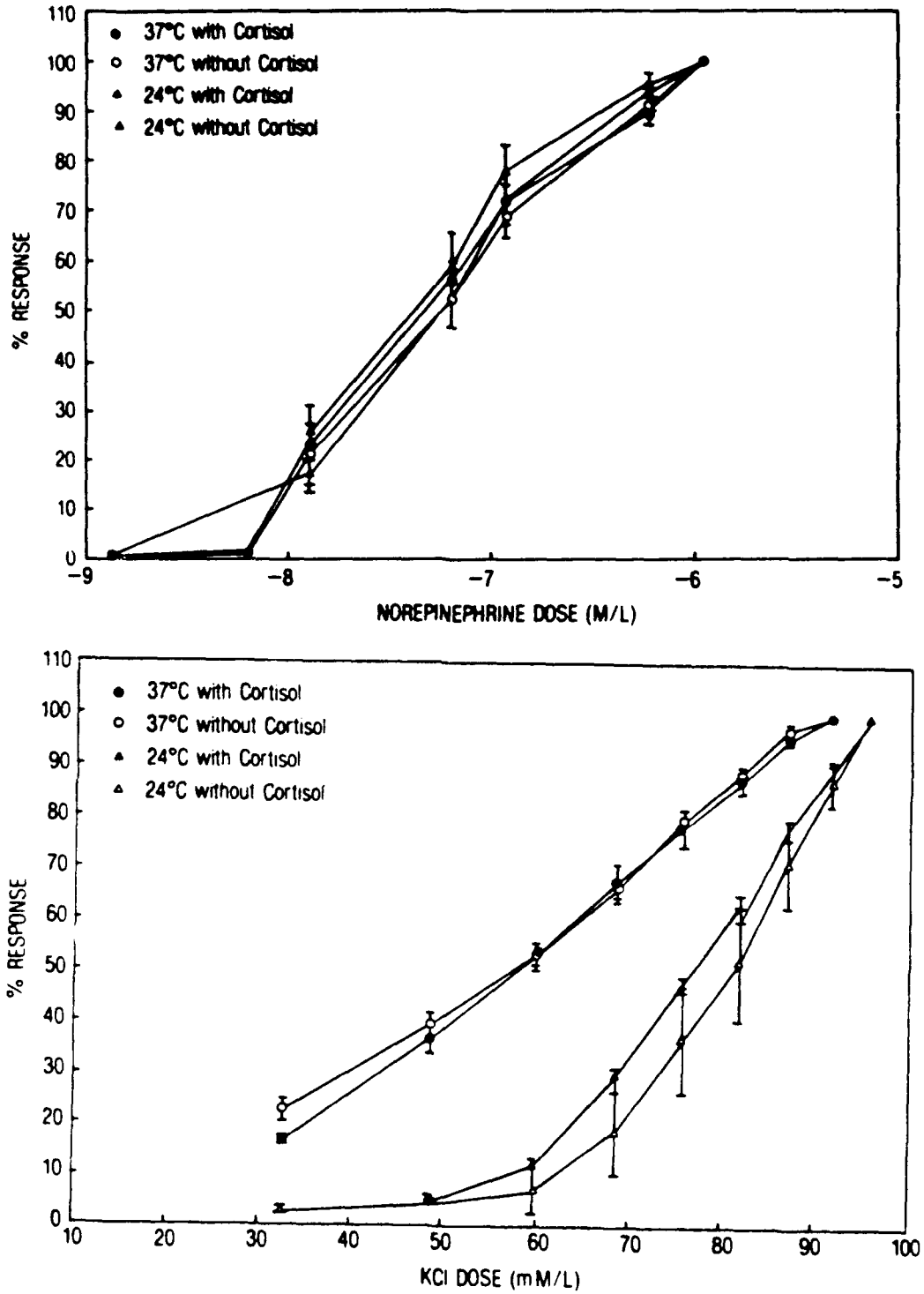

Figure 11. Upper panel. Dose-response curves of pig femoral artery rings for receptor mediated agonist at two temperatures with and without stress steroid. Lower panel. Dose-response curves of pig femoral artery rings to non-receptor-mediated agonist conducted at two temperatures with and without stress steroid. Values are means ± SEM for 3-5 arteries.

DISCUSSION

Some investigators consider the vascular sensitivity to cold to be mediated via the sympathetic nervous system (6,7,24). There are also reports of both cold-induced vasoconstriction (1,27), cold-induced vasodilation (2,14,24) and cold-induced alternating periods of both (24,28). There are several reports which describe an adaptive response of the peripheral vasculature to cold exposure (29,30).

The changes in arterial sensitivity to NEPI, which are induced by cold exposure, vary according to a number of governing factors (31,32,33). The location of the arterial bed is thought to influence the cold-induced sensitivity to NEPI (4,24), and peripheral arteries are thought to be much more labile in their cold-induced NEPI sensitivity than are deeper arteries (8). Moreover, vascular beds such as those found in the skin circulation, which are well-endowed with arteriovenous anastomoses, are highly influenced (constrict) by cold exposure (3,6,9).

In addition to these site-specific sensitivities, there are changes in the interstitial milieu which affect the disposition of neurotransmitters. One such change may be caused by elevated levels of cortisol, which reduces the uptake of catecholamines (26). Elevated levels of circulating catecholamines themselves, may also contribute to increased sensitivity by lowering the vascular threshold setting for response to NEPI. Some remote site or tissue might exert extravascular control over vascular sensitivity to NEPI. To characterize some or all of these applicable mechanisms, we investigated the sensitivity of vascular tissue to NEPI both in vitro and an in vivo.

IN VITRO EFFECTS

Contrary to some reports (8,31), the present study conclusively demonstrates that the response of various porcine or lagomorph arteries to either receptor-specific (NEPI) or non-receptor-specific agonist (KCl) (porcine only) decreases with bath temperature. In fact, the agonist-induced response disappears entirely at temperatures less than 15° C. This loss of response may be due to the well-documented decreases in cellular metabolism which occur as a function of low temperature or due to a decrease in inward calcium current, which is known to occur at very low environmental temperatures (34). A decrease in release of intracellular calcium from storage sites such as the sarcoplasmic reticulum could also occur.

A loss of membrane fluidity or a change in the phase of the lipid bilayer at low temperatures could produce this change (33). Such a change might restrict the movement of ion channel proteins in the plasmlemma, thus reducing the depolarizing K^+ current or the contraction-producing inward calcium current. It might also restrict the movement of membrane receptor proteins required for agonist-contraction transduction.

When arterial tissue was put under passive tension and exposed to cold temperatures, it relaxed significantly. Such relaxation may have been caused by a cold-induced interruption in the transduction of the agonist signal into a contractile event. It is possible that the cold-induced reduction in cellular metabolism may have facilitated hydrolysis of ATP and inhibited subsequent myosin light chain phosphorylation, steps which are necessary for the cross-bridge recycling (35) required to support the maintenance of the passive tension.

One technical improvement in the present study, which may explain differences in experimental results between this and other studies, is the constant re-setting of the pre-load to maintain opitimal position for cross bridge formation as tissues were progressively cooled. This was accomplished by programmed microprocessor control over pre-load. That is, when incubation temperature was changed, and the tissue equilibrated at the new temperature, the pre-load was automatically reset and maintained until contractile agonist was added to the bath. We are unaware of any such precise control over pre-load in other studies of the vascular contractile response at various incubation temperatures.

In light of other research with rabbits (15), it was surprising that hypothermic-derived porcine arterial tissue was no more sensitive to the in vitro administration of NEPI than was normothemic-derived tissue. Clearly, some species-specific difference in the control of arterial smooth muscle sensitivity to NEPI exists between rabbits and pigs. In rabbits, the increased sensitivity to NEPI lasts for nearly 24 hours, regardless of the extent of washing the tissue (15). A sustained event has been induced in the rabbit vascular tissue. This did not occur in hypothermic tissue derived from pigs.

IN VIVO EFFECTS

The dramatic increase in NEPI sensitivity of intact hypothermic arteries indicates that an intact vasculature is required to effect the change in sensitivity. An obvious factor is the anatomic connection in the efferent limb of the sympathetic nervous system. This has been described for other preparations, and has been suggested as an essential element for the

response in man. However, the response of the sympathetic nervous system is described as one of "mass effect" (i.e., all or none). There were no other signs of sympathetic nervous activity at the time of the increased arterial sensitivity to NEPI. Heart rate was unchanged and there was no concommittant rise in pressure in the contralateral limb.

It is possible that easily observable mechanical events (i.e. increases in heart rate and myocardial contractility) were not precipitated by moderate sympathetic activity. It is likely, however, that some tropic influence of the intact sympathetic innervation permitted, enhanced or facilitated the increase in sensitivity to exogenously applied neurotransmitter.

CORTISOL EFFECTS

Prolonged exposure to cold (sufficient cold to lower core temperature to 25° C) is a perceptible stress for the pig, and cold-induced increases in stress steroid are well documented (25). As a result, the stress of cold exposure not only triggers a sympathetic nervous system response, but also promotes the release of cortisol as well. Little is known about the direct effects of cortisol on NEPI-induced contraction. However, there are isolated reports that cortisol attenuated the re-uptake of NEPI (26). This action could be perceived as one which would prolong the extracellular concentration of agonist and possibly facilitate an enhanced contractile response. While this scenario is teleologically attractive, experiments conducted in other laboratories, using other agents to prolong the concentration of NEPI (i.e., cocaine), have ruled out this mechanism as the one responsible for enhanced vascular contraction in the cold (24).

CONCLUSION

Given the series of ionic and membrane-mediated events responsible for excitation-contraction coupling in vascular smooth muscle, the reported increased sensitivity to norepinephrine in a cold environment is surprising. The increased and prolonged sensitivity to agonist following hypothermia is not an artifact of the isolated vessel ring, but is also found in the intact animal. Futhermore, this increased sensitivity is manifest differently in the different species. The mechanism for these responses is unclear, but must involve changes in the membrane dynamics as well as non-vascular changes (in pigs). The prolonged nature of the reaction indicates that calcium must be crossing the membrane to stimulate and maintain contraction, but what does hypothermia do to the membrane? The next series of experiments should involve the blockade of Ca^{2+} transport and perhaps the use

of calcium free solutions. The elucidation of this mechanism could be very useful in the development of policy and methods for treatment of individuals following hypothermia.

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