Mean values and SEM, of the age in years, the mean blood pressure (MBP mm Hg), urinary prostaglandins E and F secreted per 24 h (PGEs ng/24 h and PGFs ng/24 h), sodium and potassium rejected per 24 h ($U_{Na}V$ mEq/24 h and U_KV mEq/24 h) and the ratio between Na/K, before (B. Ex.) and after (A. Ex.) extracellular space expansion by 2 l of isotonic saline, infused at the rate of 0.3 ml/kg/min in the group of the essential hypertensive patients

	n	Age (years)	MBP (mm Hg)	PGEs (ng/24 h)	PGFs (ng/24 h)	$U_{Na}V$ (mEq/24 h)	U_KV (mEq/24 h)	V (ml/24 h)	Na/K
B. Ex.	17	36 (23–62)	126±5	105±27	66±27	83±13	54±4	1483±390	2.04±0.33
A. Ex.	14	37 (23–62)	116±6	327±63	123±48	237±17	67±5	2592±677	3.73±0.44
	$p <$	NS	0.1	0.003	NS	0.001	0.025	0.001	0.005

The following parameters were measured before and after extracellular space expansion[16,17]. 1. Arterial blood pressure was measured 4 times a day. Mean blood pressures (MBP) were calculated from the diastolic (DBP) and systolic (SBP) pressures by the formula MBP = (2DBP + SBP): 3. The mean value of the 4 mean blood pressures was taken.

2. Prostaglandins. Bioassays were performed on extracts of 500 ml, from 24 h urine collections, after chromatographic separation into PGE and PGF groups[18,19]. The method used was described in details elsewhere[15]. Since PG metabolites appearing in the urine generally have less biological activity than the parent compound[20]. It might be assumed that most of the biologically active substances detected in this study were natural PGs.

3. Urinary output of sodium ($U_{Na}V$) and potassium (U_KV) are calculated by the usual methods. Electrolyte concentration was measured by flame photometer (Eppendorf).

Results and discussion. The results are summarized in the Table. A significant positive correlation was found between the PGEs secreted in 24 h and sodium excreted ($U_{Na}V$) in 24 h (Figure 1). After isotonic saline infusion, sodium excreted in 24 h was directly proportional to the increase in PGEs release following saline infusion (Figure 2). We did not find any correlation between the PGFs and sodium excreted either before or after saline infusion. The release of prostaglandins following either intravascular and/or extracellular space expansion is well known[11–14], and is confirmed by this study.

These results suggest that the major role of PGEs could consist in the regulation of the extracellular fluid, and consequently in the longterm control of arterial blood pressure, and that the deficiency in renomedullary PG synthesis, related to the evolution of essential hypertension[15], could be the cause and/or the result of the hypertensive disease. Finally the positive significant correlation found between the ratio of urinary Na/K and urinary PGEs ($Y = 0.015X + 0.53$, $r = 0.672$), could suggest an antagonistic result between these substances (PGEs) and the aldosterone system.

[16] Of these 17 patients 3 of them, did not undergo expansion.

[17] Extracellular space was expanded by i.v. infusion of 2 l of isotonic saline at the rate of 0.3 ml/kg/min.

[18] PGE and PGF group was assayed as ng of PGE_2 and $PGF_{2\alpha}$ equivalent respectively. Standard PGE_2 and $PGF_{2\alpha}$ were kindly provided by Dr J. Pike, Upjohn Company Kalamazoo Mich.

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The Action of Cycloheximide on the Action Potential and Protein Synthesis in Medullated *Xenopus* Axons

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Summary. Cycloheximide depresses maximum rate of change in membrane potential observed during the rising phase of the action potential in single medullated axons of *Xenopus*. Time course of depression is independent of cycloheximide concentration over a range that almost completely inhibits leucine incorporation into axonal proteins.

Under conditions of continuous fluid exchange, cycloheximide, a potent inhibitor of ribosomal protein synthesis, induces a gradual depression in the maximum rate of change in membrane potential observed during the rising phase of the action potential in an isolated medullated nerve fibre of *Xenopus*. Experimental results are illustrated in Figures 1 and 2.

The progressive increase in $\dot{V}$ associated with the 10 mg/l concentration of cycloheximide is quite often observed in control experiments, most of which, however, involve an unexplained rise in $\dot{V}$ to a plateau level throughout a time course of several hours.

However, depressive effects when present, can be reversed by means of a brief conditioning hyperpolarization under conditions such that the maximal value attained by $\dot{V}$ is identical with that which would have been obtained from a normal or non-depressed fibre after conditioning hyperpolarization. Supporting data were obtained from monitoring experiments similar to those involving recent investigations of taxine action[1].

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Hence it is concluded that the cycloheximide depression in question must involve a variation in some membrane conductance factor rather than a change in either the sodium or potassium electrochemical gradients. Numerical solutions of the FRANKENHAEUSER-HUXLEY equations[2,3] have indicated that these results are compatible with translations or distortions of the sodium inactivation curve and the potassium activation curve. However, excluded as theoretical possibilities are variations involving sodium activation, permeability constants or electrochemical gradients[3,4].

Results described in Figures 1 and 2 also indicate that the time course of depression in $\dot{V}$ is essentially independent of cycloheximide concentration within a range of 25 to 500 mg/l.

These findings together with those relating to conditioning hyperpolarization are then taken to indicate that the drug blocks a chain of reactions or processes at a link remote from that directly concerned with maintenance of the resting level of the conductance factor involved (probably the h factor). In such an event the disappearance of a critical membrane structural component should proceed at a rate which is independent of the cycloheximide concentration within rather wide limits.

A possible mechanism implicating interference with protein synthesis is suggested by the results shown in the Table which refer to a cycloheximide induced depression of leucine incorporation into protein of whole desheathed *Xenopus* sciatic nerves. Test nerves were first incubated for 1 h in a Ringer solution containing cycloheximide in the concentrations indicated. Both test and control nerves were then incubated for 1 h in 2 ml volumes of Ringer containing 2 μCi ^{14}C-L-leucine with a specific activity of 250 μCi/μm. Cycloheximide was present at all times in the solutions containing the test nerves. Subsequent analytical procedures were essentially identical with those of CUNNINGHAM and BRIDGERS[5]. Protein determinations were obtained by the method of LOWRY[6]. Mean percent inhibition in the Table corresponds to the mean of a set of terms each of which is given as $100\ (1\text{-}x/c)$ where x is cpm per mg of protein in test nerve and c is cpm per mg protein in contralateral control nerve.

At first glance it may seem surprizing that an 84% inhibition of leucine uptake is without effect on $\dot{V}$, whereas a 93% inhibition is associated with a maximal rate of decline in $\dot{V}$ over several hours. This implies that a rather slight reduction in the concentration of some protein component to a non-zero level would eventually result in the complete disappearance of a protein complex necessary for maintenance of a sodium conductance factor at its resting level.

Mean percent inhibition of radioleucine incorporation in nerve protein by cycloheximide

No. experiments	Concentration of inhibitor	Mean inhibition (%)	SD	SE
8	10 mg/l Cycloheximide	83.9±	5.32	1.88
4	50 mg/l Cycloheximide	92.5±	2.05	1.03
9	250 mg/l Cycloheximide	93.7±	3.13	1.04

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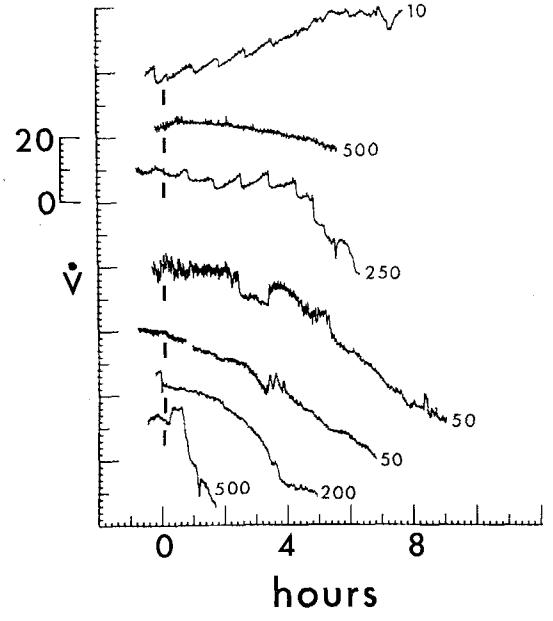

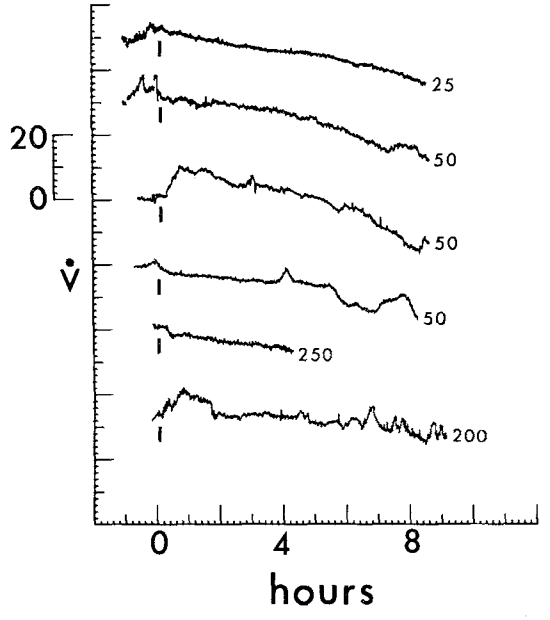

Figures 1 and 2. Ordinate is percent change in magnitude of $\dot{V}$, the maximum rate of change of voltage during the rising phase of the action potential obtained from a large single medullated *Xenopus* fibre. Each point corresponds to a mean of 9 consecutive measurements taken at 2 sec intervals. Stimulation rate is once per sec. Print out from a PDP8-I computer is effected by a Calcomp 565 X-Y recorder which prints 200 points per hour. The fibre is subjected to continuous fluid exchange. At zero time each fibre is exposed to concentrations of cycloheximide indicated by numbers adjacent to each curve in terms of mg/l. Each of the fibres involved is obtained from a different nerve. The progressive increase in $\dot{V}$ associated with the 10 mg/l concentration is quite often observed in control fibres. Recording technique involves the single air gap method.

Such a situation is, however, often encountered in a system involving cooperativity, where a fairly large number of identical components may react to form a single complex.

As an illustrative example, the reaction sequence

$$nP + S \rightleftharpoons P_nS + C \rightleftharpoons P_nSC$$

may be constructed where n molecules of a protein P combine successively with components S and C in a situation such that $[C]$ is limited and $[S]$ is present in excess. Resting level of sodium conductance might then be determined by $[P_nSC]$.

For reasonable values of n, say 5 to 20, the concentration term $[P_nSC]$ can vary with $[P]$ in a manner such that it is essentially zero throughout a quite extended range of $[P]$, beyond which, it increases abruptly and sigmoidally to approach a limiting value determined by the available C factor.

Hence it is conceivable that an abrupt reduction in $[P]$ throughout a narrow range would lead to a gradual time dependent decline in $[P_nSC]$ which might then be reflected in the $\dot{V}$ curves of Figures 1 and 2.

While amino acid incorporation into protein of isolated axons is well established for a variety of forms, the synthetic mechanism involved remains obscure[7–12]. The problem is, of course, aggravated by the absence of clearly demonstrable ribosomes in axoplasm. On separating squid axon sheath from axoplasm, LASEK et al.[13] discovered that most of the ribosomal RNA is confined to the sheath.

Exploration of these results to the medullated axon would implicate the Schwann cell as a source of ribosomal synthesis with subsequent migration of protein into the axon. Not excluded as a possibility is the suggestion by AUSTIN and MORGAN[14] that since high levels of RNA are found in synaptosomal structures it is possible that the axonal membrane itself may contain ribosomal types of synthesizing systems.

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Alpha-Receptor Subsensitivity of Isolated Atria from Rats Following Repeated Injections of Phenylephrine or Isoprenaline[1]

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Summary. Daily injections of phenylephrine or isoprenaline for 4 or 7 days lowered the chronotropic sensitivity of α-adrenoreceptors in isolated rat atria.

An increased sensitivity of cardiac α-receptors is found to be induced in frogs and/or mammals by several conditions, e.g. by low experimental temperature[3–7], inactivity of skeletal muscles[8], increased vagal influences on heart[9], propylthiouracyl treatment[10,11], and hypothyroidism[12]. These effects are usually associated with a lowered β-receptor sensitivity shown in inotropic and/or metabolic activity of this organ. Recently we[13] found out that cold acclimation temporarily lowers chronotropic sensitivity of the isolated rat atria to an α-adrenergic drug phenylephrine (PHE), while it does not change the sensitivity to a β-adrenergic drug isoprenaline (ISO). These results were interpreted by suggesting that the enhanced release of catecholamines, as a result of increased sympathetic activity of cold-exposed animals, was responsible for this subsensitization of cardiac α-receptors.

The aim of the present study was to elucidate whether increased levels of α-stimulant (PHE) and β-stimulant (ISO) in rats, as produced by repeated injections of these drugs, will change the sensitivity of cardiac adrenoreceptors in isolated atria.

Material and methods. A total of 198 adult male Sprague-Dawley rats, 200–350 g in weight, were used in these studies. They were divided into 20 groups, half of them receiving once a day subcutaneously injections of PHE (Neosynephrine®, Winthrop) 3 mg/kg in physiological NaCl-solution or ISO (Isuprel®, Winthrop) 0.3 mg/kg in olive oil. The duration of the treatments in each group is mentioned in the Figures. The other half served as control animals receiving injections of NaCl-solution or olive oil without any drugs.

The cumulative concentration-response curves for the chronotropic response to ISO and PHE were determined on isolated atria at 37 °C in Thyrode's solution. The

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